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RATE OF GROWTH, AGE AT SEXUAL MATURITY, AND DURATION OF LIFE OF CERTAIN SESSILE ORGANISMS, AT WOODS HOLE, MASSACHUSETTS

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While studying the breeding habits and rate of development of *Teredo* the writer noted the very rapid growth of several sessile organisms, notably campanularian hydroids, *Hydroides*, *Balanus*, *Botryllus*, and *Molgula* which became attached to the wooden floats that were placed in the water in connection with the above study. No doubt the remarkably rapid growth of many animals of this sort has been observed by all who interest themselves in sea life. There are, however, comparatively few accurate data on the rate of development of these organisms and their age at sexual maturity. Studies of embryology have usually ended with organogeny rather than with life histories.

Without definite data it would not have been suspected that numerous organisms reach sexual maturity in three or four weeks or that the rate of multiplication and of growth is so remarkably rapid as it has proved to be. These facts contribute to an understanding of the sea as a reservoir of life and the methods by which this life is maintained.

In this paper the discussion of each species has been stated in the briefest possible form because we are here interested only in the outstanding facts of their life histories including the limits of the breeding seasons, rate of growth, duration of life, and number of generations per year. The study as a whole is intended to give a correct idea of the productivity of the organisms included. In this way a foundation is laid for an understanding of the interrelation of organisms and for greater accuracy in ecological or experimental studies.

This investigation was done at the Marine Biological Laboratory at Woods Hole and has covered a period of approximately ten years. During two years observations and measurements were made during

every month in the year. Aside from this the work was confined to the summer months.

I wish to acknowledge my indebtedness to Dr. M. H. Jacobs, Director of the laboratory, and to the permanent staff for assistance and for facilities furnished during the course of the investigation.

Methods

In studying the rate of growth of organisms certain materials and devices were finally selected to serve as places of attachment for sessile organisms. Among the most useful of these were wooden crosses constructed of 2″ × 4″ timber. These were placed in the Eel Pond and anchored to piles at dated intervals. The approximate date on which animals became attached could thus be known and the records of rate of growth kept with accuracy. The entire life history of individual animals could be followed or samples could be studied at intervals. Other materials that proved useful were 2″ × 4″ stakes ten or twelve feet long, one end of which was sharpened and driven into the mud while the other was nailed to a pile at the surface of the water. These and lobster pots made of smaller timbers proved to be most useful for the study of *Teredo*, but they served also as places of attachment for several other animals. Shells of mollusks, small stones, and bricks were sunk in wooden cages to serve expecially as attaching material for *Hydroides*. Finally, small wooden frames with grooves for holding microscope slides or small wooden strips served as materials easily examined by the microscope. These last were particularly valuable in observing early stages of metamorphosis and growth. Most animals which attached to microscope slides grew normally, but more slowly than when they attached to other materials. The floating wooden crosses were by far the most useful for the study of hydroids, barnacles, Bryozoa, and Ascidia. The sunken timbers were for *Teredo* and the stones and shells for *Hydroides*. In the discussion which follows, frequent reference will be made to the floating wooden crosses constructed as described above.

Coelenterata

Most hydroids thrive best during spring and fall, although there are a few that do not reproduce until June. One or more of the four species studied were reproducing every month in the year at Woods Hole, with the possible exception of January. They, together with certain algae, remain active and growing throughout the winter and are clearly in healthiest condition while the water is cool. Although the growth of hydroids was comparatively slow during February,

March, and April, the colonies and individual polyps appeared to be particularly healthy. They were free from debris and bacterial growth which so completely smothers them during July and August in such situations as the Eel Pond. They harbored a few stalked diatoms which themselves were clean and apparently no detriment. Chief among the enemies of hydroids at this time, as mentioned also by Coe, are nudibranch mollusks which feed upon the exposed polyps. From the middle of July until the middle of August hydroids are generally in very bad condition. The excessive growth of bacteria during the hottest part of the summer no doubt contributes to their hardships although they are distinctly cold water animals and are affected adversely by high temperature.

Two of the species of *Campanularia* studied, *C. flexuosa* and *C. calceolifera*, reach sexual maturity in four weeks under the most favorable conditions. This is also true of *Gonothyrea loveni*. All of them, under usual conditions, are reproducing abundantly in five weeks. The largest species studied, *Obelia commissuralis*, grows rapidly for six to eight weeks before reproducing sexually. Its colony when fully grown is four to five inches in length and consists of hundreds of polyps in contrast to the smallest species which have from twenty to fifty polyps. They all reproduce most abundantly in April and May and continue with little check until the middle or end of June, when they seem to disintegrate and although some new colonies are initiated throughout the summer, they are all in poor physiological condition. Late September and October are also periods of rapid growth. As cold weather comes on the rate of growth is reduced but the polyps remain active and new colonies continue to be established.

Although hydroids are not large animals they are so abundant that they foul all timbers at or near the surface during most of the year. Together with *Bugula* and *Botryllus*, which are abundant in summer and fall, they effectively cover all surfaces in a few weeks.

The planula larvae of species studied swim for a few hours and then become attached. Under favorable conditions they develop into feeding polyps within twenty-four hours. At this time they are already elongated and the hydrocaulus has a distinct perisarc including characteristic joints near the base. The rate of increase in growth of the colony varies with the species and with the temperature. In the following table the rate of growth of *Obelia commissuralis* is given. It shows that the number of polyps of the growing colony doubles every two or three days. After five days the first polyp which arises from the planula may have budded to a colony of five to seven feeding individuals, and in nine days there are twenty to forty polyps. The

colony consists of hundreds or even thousands of individuals before it becomes sexually mature. The polyps were not counted in any colony more than a month old when it measured three inches in height and consisted of more than five hundred individuals.

So far as sexual reproduction is concerned, the smaller species proved to be actually more prolific than this species. They produce gonosomes early and the numbers of planuli produced by them during April and May is remarkably great.

The normal duration of life of hydroids varies with the time of year, but very few colonies survive the summer. The old colonies are so rapidly replaced by younger ones that it has been a difficult matter to get definite data in regard to length of life. It is certain that they live for several months unless difficult conditions arise. They certainly do not survive for a year. The total amount of growth is, however, great in situations favorable to their development.

TABLE I

Rate of growth of Obelia commissuralis. The left-hand column gives the period of growth beginning with the attachment and metamorphosis of the planula. The column at the right indicates the year. All measurements are of actual colonies and are not averages.

Period of Growth	Age	Height	Colony	Year
		mm.		
June 24	0 day	.35 × .10	Planula	1926
June 24 to June 25	1 day	.8 × .07	1 polyp	1926
June 20 to June 25	5 days	2 to 3	5 to 7 polyps	1932
June 19 to June 27	8 days	8.5 to 10	28 to 40 polyps	1931
June 9 to June 18	9 days	14 to 17	26 to 42 polyps	1931
June 9 to June 19	10 days	15 to 17	50 to 65 polyps	1931
June 20 to June 30	10 days	19 to 20	55 to 65 polyps	1932
June 9 to June 22	13 days	22 to 26	140 to 160 polyps	1931
June 10 to June 24	14 days	30	140 to 160 polyps	1932
June 9 to June 27	18 days	55 to 57	500 to 560 polyps	1931
April 8 to May 18	40 days	45 to 50		1927
Aug 12 to Oct. 12	60 days	65 to 70		1926
Oct. 10 to Jan. 3	86 days	100 to 120		1926
Jan. 1 to May 15	4½ months	50 to 55		1926 and 1927
Sept. 20 to May 15	Winter	55 to 65		1926

CRUSTACEÆ—BALANUS EBERNEUS

Of the several species of barnacles that occur at Woods Hole, *Balanus eberneus* is the only one which thrives in the Eel Pond, where this study has been conducted.

The eggs are retained in a brood cavity during early development and the young issue as nauplius larvæ which swim actively. These

have a free-swimming period of approximately a week or ten days during which they moult several times and pass through the transformation stages known as nauplius, metanauplius, and cyprid. The cyprids finally settle upon solid objects such as stones, piles and wooden structures and metamorphose into the adult form. The present study is concerned principally with the breeding season and rate of growth from metamorphosis to adult size. My conclusions concerning the duration of the free-swimming nauplius are based upon the observation that after the first appearance of swimming larvæ only a week elapses before metamorphosis and permanent attachment of the cyprids take place.

TABLE II

Rate of Growth of Balanus eberneus

Period of growth	Age	Length	Breadth	Year
		mm.	*mm.*	
July 19 to July 25	6 days	.8	.7	1932
Aug. 8 to Aug. 17	9 days	2	1.7	1929 and 1932
June 27 to July 18	10 days	2.3	2.	1930
July 15 to July 27	12 days	2.8	2.3	1926
July 1 to July 15	14 days	3.5	2.8	1926
Aug. 4 to Aug. 18	14 days	3	3	1929
June 24 to July 13	16 days	4	3.5	1930
July 25 to Aug. 12	18 days	5.2	4	1926
July 15 to Aug. 12	28 days	7.4	6.5	1926
June 20 to July 22	32 days	10	9	1932
June 22 to July 29	32 days	9	8	1927 and 1931
July 15 to Aug. 20	36 days	10	9	1926
June 27 to Aug. 2	36 days	11	10	1930
June 14 to July 22	38 days	12	11	1932
July 9 to Aug. 23	44 days	13	12	1927
June 27 to Aug. 15	49 days	15	14	1932
June 27 to Aug. 15	49 days	15	13	1926
June 27 to Aug. 20	54 days	16	15	1930
June 27 to Aug. 20	54 days	17	16	1931
June 23 to Aug. 23	60 days	15	14 *	1927
June 23 to Aug. 23	60 days	14.5	12.5*	1925
July 15 to Sept. 20	67 days	17	13	1926
June 27 to Sept. 7	76 days	15	14	1927
June 20 to Sept. 4	70 days	19	16	1931
July 1 to July 1	1 year	20.5	19.5	1927
July 1 to July 1	1 year	18	16	1926

* Sexually mature in 60 days and over.

According to data collected over a period of years the first larvæ of the season escape from the parent between June 7 and June 15. The first cyprids become permanently attached and metamorphose between June 15 to June 23. The first young barnacles appear visible

to the unaided eye between June 23 to July 4. Ten days after metamorphosis the young barnacles measure 2 × 1.5 mm. and are readily seen upon the wooden floats used in this study.

From the time the cyprid larva metamorphoses into the barnacle the growth is rapid as shown by Table II. Sexual maturity is reached in two months (60 days) and the first nauplius larvæ issue from young barnacles of the season between August 20 to August 25. For example, a float placed in the Eel Pond on June 23 had barnacles attached which extruded nauplius larvæ on August 23. There are considerable numbers of barnacle larvæ in the water from August 25 to October 1, but the fall breeding season amounts to little compared with the June and July breeding season of this species.

Growth practically ceases late in October and is resumed the following spring.

At the end of one year this barnacle measures from 18 × 16 mm. to 21 × 19 mm. depending upon conditions, including crowding. They apparently reach maturity during the second year. No individuals have been kept under observation for a longer period than one and one-third years.

Although this species of barnacle breeds more or less all summer, the important part of the period of fouling extends from June 25 to July 25, or from June 25 to July 15. By far the greatest amount of fouling of timbers, therefore, occurs during a period of three weeks. Those attaching after July 25 are negligible. This was shown to be true also of a winter breeding species. The cyprids of *Balanus balanoides* metamorphose in greatest numbers between February 15 and March 15, and although they continue to shed a few nauplius larvæ until May, the breeding season is mainly over in four weeks.

In general, barnacles reach sexual maturity early and are short-lived as compared with many other crustaceans.

Annelids—Hydroides hexagonis

This species of annelid secretes a calcareous conical tube which adheres to shells of mollusks, stones, and wooden structures.

The breeding season opens between June 10 and June 20 and closes between October 20 and November 1. The egg has an unusually heavy vitelline membrane. It measures from .067 to .072 mm. when fully mature. During the early part of the breeding season over fifty per cent of the spawned eggs are immature and undersize. Toward the end of the breeding season the eggs are all mature, indicating that the germinal epithelium is becoming inactive. Maturation of the egg does not take place until the spermatozoön enters. The germinal

vesicle measures half the diameter of the egg or (.03 mm.). The sexes are separate. To obtain eggs or sperm, it is advisable to remove the worms from their calcareous tubes and place them in dishes of sea water, one worm per dish. When so treated they always spawn immediately. The spermatozoa when first extruded are immobile but become slightly activated after remaining in sea water for approximately half an hour. It is a relatively inactive sperm at best. After fertilization the egg develops within twenty hours into a spherical transparent trochophore larva, which swims for at least ten days before metamorphosis.

It finally and gradually develops a slender worm body, settles and secretes a calcareous tube. After this the worm is immovably fixed to some supporting object. In studying the rate of growth the increase in the size of the worm tube is taken as the index of the growth of the worm. The shell, of course, grows and extends in length from its open mouth. It is not a difficult matter to study the growth of a single worm, but in practice the largest worms of certain ages were measured, and no attempt was made to record the growth rate of a single worm. The data for the several years of study is fully consistent except that the growth rate for the years 1924–25 is not equal to that observed during the past three years.

Table III gives the approximate rate of growth after metamorphosis.

TABLE III

Rate of Growth of Hydroides hexagonis

Period of growth	Age	Length and breadth	Year
		mm.	
Aug. 6 to Aug. 22	16 days	7 × .6	1931
July 20 to Aug. 10	21 days	11 × .6	1927
July 20 to Aug. 15	25 days	13 × .8	1931
July 20 to Aug. 20	30 days	20 × 1.2	1931
Aug. 2 to Sept. 3	33 days	13 × 1	1931
June 20 to Aug. 2	36 days	25 × 2	1924
June 27 to Aug. 11	44 days	37 × 2.2	1931
June 27 to Aug. 15	49 days	45 × 2.3	1931
June 27 to Aug. 25	59 days	54 × 2.7 *	1931
Oct. 18 to May 6	Winter	5 × 1.1	1926
Aug. 20 to Aug. 20	1 year	66 × 3.0	1924 and 1925
	2 years	120 × 5	1927

* Sexually mature in 59 days.

The worm becomes sexually mature in seven or eight weeks after metamorphosis, and before it is nearly half grown. The first eggs from these young worms are spawned about August 20, and egg production

continues until about October 25 in both young and old worms. The larvæ of this species are metamorphosing and worm tubes are being formed during the entire breeding season. The worm becomes fully grown in two years and probably does not live beyond that age. The tubes of the largest worms measure 12 cm. in length and 5 mm. in greatest diameter. A worm 10 cm. long and 4.5 mm. in widest diameter is a large worm and is nearly the average size at maturity. The largest of those kept for one full year measured 7 cm. in length and 3 mm. in widest diameter, which may be considered average. It should be said that no worms were kept under observation for more than fifteen months. Because they have not attained full size in that time the impression was gained that individuals survive for two years and not much beyond that. Most of the worms ordinarily collected are only one year old. Comparatively few survive to reach the largest size recorded in Table IV.

TABLE IV

Rate of Growth of Botryllus gouldii

Period of growth	Age	Size of colony	Number of individuals of colony	Year
		mm.		
July 19 to July 22.....	3 days	?	One gonozoid complete	1932
July 19 to July 24.....	5 days	1.5 × 1.5	Gonozoid and first blastozoid complete and initiation of further budding	1932
July 19 to July 26.....	7 days	4 × 4	4 to 5 complete individuals and initiation of further budding	1932
July 19 to July 29.....	10 days	5 × 5	8 to 10 individuals	1931
July 20 to July 31.....	11 days	6 × 6	18 to 20 individuals	1931
July 20 to Aug. 3.....	14 days	10 × 7	30 to 45 individuals	1931
July 20 to Aug. 5.....	16 days	12 × 10	52 to 58 individuals	1931
Aug. 6 to Aug. 30.....	24 days	30 × 20	200 to 300 individuals	1927
Aug. 6 to Sept. 6.....	30 days	50 × 30	1000 individuals	1927
June 20 to July 20....	30 days	90 × 50*	3000 individuals	1931
Oct. 10 to Nov. 26....	Fall	5 × 5	6 to 8 individuals	1926
Oct. 26 to Jan. 3.....	Fall	5 × 5	6 to 10 individuals	1926 and 1927
Oct. 26 to May 15.....	Winter and spring	12 × 12	50 to 75 individuals	1926 and 1927

*Sexually mature in 30 days and above.

ASCIDIANS—BOTRYLLUS GOULDII

The compound ascidian *Botryllus gouldii* has proved to be extremely variable in rate of growth. In compiling the table on rate of

growth I have arbitrarily selected the figures which show the most rapid development observed in the eight years study. The data are taken mostly from observations of the same year. The table shows that the number of individuals of the growing colonies doubles every two or three days. Under favorable conditions a colony starting from a tadpole may consist of one or two thousand individuals in a month. The measurements as given are of representative colonies and not averages.

During August and September all floating and slightly submerged structures may be completely covered by the colonies of this species Growth during the cooler months slows down to insignificant proportions. The breeding season begins sometimes as early as May 15 or sometimes not until June 10. It continues until November 1. *Botryllus* has a long breeding season, but is most prolific during July, August, and September. The colonies reach sexual maturity and extrude tadpoles in from 35 to 50 days after metamorphosis. A wooden float placed in the water on June 20, 1931 developed colonies from single tadpoles which measured 9 by 5 centimeters, containing 3000 individuals on July 20. These colonies which were thirty days old contained mature eggs and on their thirty-fifth day, July 25, they extruded tadpoles. Another colony which developed on a float which was placed in Eel Pond on June 27 extruded swimming tadpoles on August 3. Another starting on May 15 became sexually mature on July 4. The species therefore reaches sexual maturity under the most favorable conditions in five weeks. Many large colonies survive and are active all winter, contracting perceptibly when stimulated. However, a large percentage must die during the winter because in the spring there are no such numbers surviving as covered piles and floating structures during late summer. The chief fouling of these timbers occurs from June until October, after which the rate of growth and of reproduction is greatly reduced or practically ceases. Growth revives in March and reproduction in May. A few individuals surviving the winter might literally populate to capacity the adjacent parts of the sea suitable to their existence before the next winter. In establishing the colony the metamorphosing tadpole soon develops into a sessile individual, termed a gonozoid, similar to a minute molgula. The colony is then produced by characteristic and repeated budding, the details of which have recently been reinvestigated by Sister Florence Marie Scott at Woods Hole (not yet published).

Molgula manhatensis

The growth of the solitary ascidian, *Molgula manhatensis*, may be given briefly by way of comparison with the colonial form. The as-

cidia are hermaphroditic and the eggs of this species are laid and fertilized at night. They develop within twenty-four hours into swimming tadpoles which soon become permanently attached. The attached tadpole metamorphoses promptly into the adult form and rapid growth ensues. When the molgula is two weeks old the gonads are visible through the translucent test. At three weeks of age a few mature eggs are shed and a larger number at a month. *Molgula manhatensis*, therefore, under favorable conditions reaches sexual maturity in three weeks or a month, at which time it measures approximately 10 × 8 mm. It spawns abundantly during its first season. A large specimen measures 23 × 22 mm., a size which may be attained before winter. To secure eggs, place sexually mature specimen in a dish of sea water and allow to stand undisturbed. Eggs will be shed normally during the evening. They may also be had at any time by dissecting the gonads of sexually mature specimens.

Bryozoa—Bugula flabellata

The numerous species of Bryozoa which occur along the Massachusetts coast are similar in the rapidity with which they grow and come to sexual maturity. Several generations are produced every season.

The breeding season of *Bugula flabellata*, taken as an example, opens between June 1 and June 15 and closes about November 15. With the exception of hydroids and possibly one or two ascidians, the Bryozoa are the only sessile animals that have been found breeding at Woods Hole after November 1.

Most of the larger colonies of *B. flabellata* are killed during the winter but the small ones survive and the species is abundantly reestablished in June. Sometimes the first new colonies are seen during the first week in June and sometimes not until the third week. Following a hard winter the breeding season opens late, which is correlated with the killing of the larger colonies.

Colonies of *Bugula flabellata* which are established in June reach sexual maturity in one month and old age in three months. Those which are established during October may survive for ten months including the winter, which is a period of hibernation. The life history of this species, including a table on the rate of growth, has been published (Grave, B. H., 1930). The only alterations to be made in that account are that the first avicularium appears on young colonies of four functional individuals rather than later, and when the colony has grown to eight functional individuals there are avicularia on the second, third, and fourth members of the colony. As stated before, the first or attaching member of the colony never develops an avicularium.

The hold-fast very early becomes branching and stolon-like rather than disc-like as stated in that paper.

The embryos of *Bugula flabellata* are developed in brood pouches or ovicells. The larvae are shed at dawn or early morning.

To secure swimming larvæ several mature *Bugula* colonies should be collected late in the afternoon and placed in a dish of sea water. Larvæ escape from these colonies on the following morning, often by hundreds. After swimming from four to six hours they become attached and the colony rapidly develops.

Mollusca

Observations on three species of mollusca, *Teredo navalis*, *Cumingia tellinoides*, and *Chætopleura apiculata*, form an integral part of this study, but in each case separate articles have been published, which give the life histories of these forms in detail (Grave, B. H., 1928 and 1932). Mention should be made that *Teredo navalis* becomes sexually mature in two months and reaches adult size in one year: *Cumingia tellinoides* and *Chætopleura apiculata* become sexually mature in one year and reach adult size in four years.

Summary

Although *Balanus*, *Hydroides* and *Teredo* become sexually mature and spawn during their first season when they are only two months old, they spawn most abundantly during their second season when they are one year of age. Each of them spawns several times during the second summer.

Bugula, *Botryllus* and various hydroids, on the other hand, spawn principally before they are two months old and it is doubtful if they ever survive for a full year. Some of the most prolific animals seem not to live more than a year and they owe their great abundance to their production of several generations in a single summer.

These characteristics of organisms in a measure account for the abundant life of the sea and of the rapid rehabilitation of certain species after depletion. Although the sessile animals studied do not survive for more than one or two years such animals as *Cumingia*, *Chætopleura*, and *Ostrea* often live from four to six years and spawn several times each year.

The surprising discovery of this study was the great rapidity of growth and early sexual maturity of these organisms.

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THE ACTION OF ERGOTAMINE ON THE CHROMATOPHORES OF THE CATFISH (AMEIURUS NEBULOSUS)

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It is a well-known fact that ergotamine paralyzes the action of adrenalin and the effects of sympathetic stimulation. But ergotamine has also a definite action on the denervated cells, and this action, as far as it has been investigated, is the same as that of adrenalin. Thus ergotamine, in the cat, increases the rate of the denervated heart (Cannon and Bacq, 1931), contracts the denervated nictitating membrane (Rosenblueth, 1932 and personal observations), contracts the retractor penis muscle (Bacq, 1933), excites the secretion of the denervated sweat glands (Fröhlich and Zak, 1932 [2]), increases the blood pressure after complete sympathectomy (Bacq, Brouha, and Heymans, 1932).

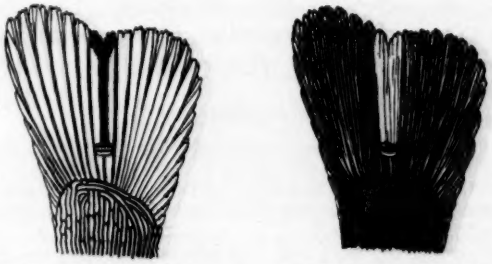

FIG. 1. Sketches of the tail of a catfish six days after cutting, before and after the injection of ergotamine.

Adrenalin contracts the melanophores of the fishes, either in normally innervated areas or in denervated regions. I found that ergotamine expands the chromatophores in the normal skin and contracts these cells when denervated. Catfishes (*Ameiurus nebulosus*) were kept in a white illuminated dish. A large cut was made in the tail according to the technique extensively used since Wyman (1924). A dark band due to the expansion of the chromatophores developed pos-

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[2] These authors have found that adrenalin excites the secretion of the denervated sweat glands. I have been able to confirm their observations (Bacq, 1933).

terior to the cut. Six days after the operation, only the central portion of this band and a triangle at the posterior edge of the fin were dark. At that time, 0.25 mg. of ergotamine tartrate (Gynergen Sandoz) injected in the muscles would evoke after ten to fifteen minutes a complete darkening of the animal with the exception of the denervated area of the tail which appeared perfectly light. The dark band and triangle were light colored and indistinguishable from the rest of the denervated area. This condition persisted for two or three hours (Fig. 1).

These observations show that ergotamine expands the normally innervated chromatophores and that it contracts the same cells when denervated. This difference in reaction occurs also in the mammal. For instance, ergotamine decreases the blood pressure of the normal cat, but produces in this animal when sympathectomized a tremendous hypertension. This denervation of the smooth muscles of the arterioles shows a direct action of ergotamine on these cells.

Conclusion

Ergotamine expands the innervated chromatophores of the catfish and contracts the same cells when denervated. This effect on the innervated chromatophores is opposite to that of adrenalin, but the direct action of ergotamine and adrenalin on the denervated areas is similar.

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EFFECTS OF CENTRIFUGAL FORCE ON FERTILIZED EGGS OF ARBACIA PUNCTULATA AS OBSERVED WITH THE CENTRIFUGE-MICROSCOPE

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By means of the centrifuge-microscope of Harvey and Loomis (1930), it is possible to observe continuously the effects of centrifugal force on eggs at various stages of development, while they are being rotated. A striking difference in behavior of the fertilized and unfertilized eggs of *Arbacia* is immediately apparent when studied in this way—a difference connected with changes in the elasticity of the fertilization membrane, in the egg surface, and in the viscosity of the egg itself. Behavior of the unfertilized eggs has been previously described (E. B. Harvey, 1932, 1933), and this paper presents the phenomena to be observed after fertilization. The experiments were performed at a temperature of approximately 23° C. Observations have also been made on eggs centrifuged in tubes where higher speeds could be obtained.

Fertilized Eggs

When *Arbacia* eggs are fertilized and placed as quickly as possible (30–90 seconds) in the special slide for the centrifuge-microscope (or in tubes), above a layer of 0.95 molal sucrose solution (which keeps them suspended) and subjected to a centrifugal force of 5000 times gravity, they break apart very readily into halves and quarters (Photograph 1). If broken apart (in tubes at 11,000 times gravity) before the elevation of the fertilization membrane, this membrane may subsequently form over each part, but is thinner (ruptures more easily) over the centripetal pole of the part; it is also thinner over the oil cap of the elongate whole egg. These parts are similar to those from unfertilized eggs (E. B. Harvey, 1932), but in most batches of eggs the elongation of the egg is greater and the halves and quarters are produced much more rapidly, as shown by comparison with a control unfertilized lot centrifuged at the same time (Photograph 2). This must be due to a decrease in the tension of the surface and not to a decrease in the viscosity of the egg protoplasm, since *the fertilized eggs at this stage and at every other stage stratify less rapidly than unfertilized eggs*. This fact can be very accurately determined by observation of

the two sets of eggs at the same time while rotating, in the special double-head centrifuge-microscope devised by E. N. Harvey (1933). The difference in stratifying between unfertilized eggs and eggs immediately or shortly after fertilization is very slight, but becomes very marked in later stages, as observed by Heilbrunn (1915, 1928) and Chambers (1919).

For about five minutes after fertilization, the fertilization membrane, which appears one and one-half to two minutes after insemination, is easily broken by the centrifugal force; and during this period (one and one-half to five minutes after fertilization) the eggs break very readily into many small pieces (Photograph 3 as contrasted with the unfertilized control in Photograph 2). Here again the viscosity as determined by stratification is less in the unfertilized egg.

After five minutes the fertilization membrane becomes so tough that it does not stretch and permit the eggs to elongate and break apart. It is possible, however, to remove the fertilization membranes as soon as they are formed by shaking the eggs quite violently for a moment. When the eggs from which membranes have been removed are centrifuged from five to twenty minutes after fertilization, during the monaster stage, they stretch out enormously, forming long streamers connecting clear and yolk portions of the egg. They stretch the whole length of the field, sometimes becoming ten times as long as the original diameter of the egg (Photograph 4). When these streamers break, they form beaded chains which retract into tails. If the centrifuge is

PLATE I

PHOTOGRAPH 1. Fertilized eggs centrifuged 30 seconds after fertilization; note greater elongation of yolks and greater number of eggs pulled apart as compared with the control unfertilized eggs centrifuged at the same time (Photograph 2).

PHOTOGRAPH 2. Control unfertilized eggs centrifuged at same time and rate as Photographs 1 and 3.

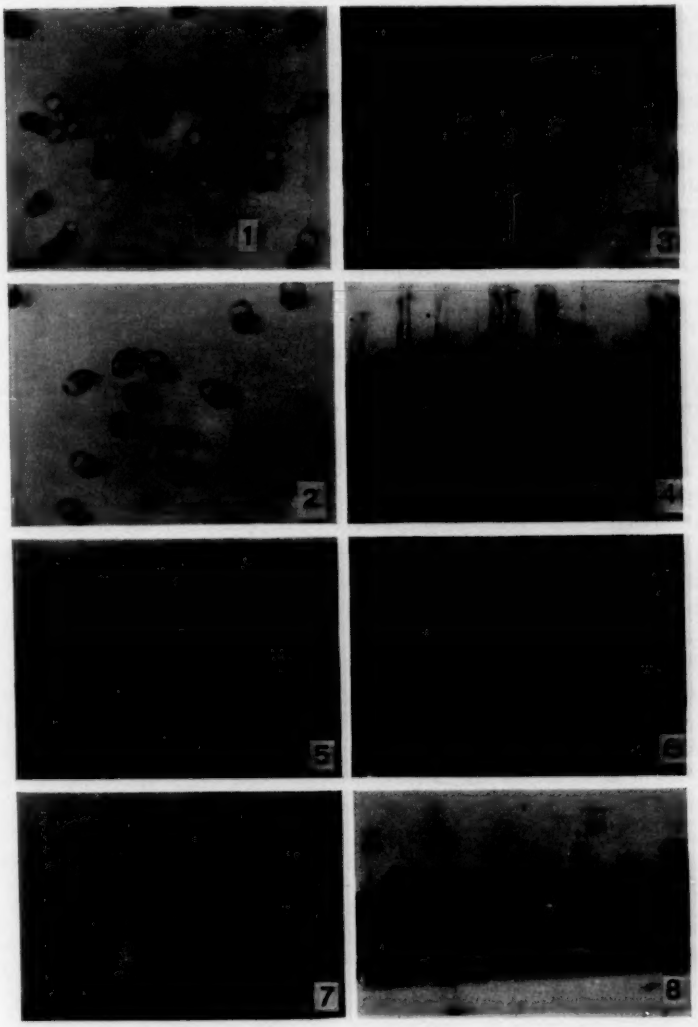
PHOTOGRAPH 3. Fertilized eggs centrifuged 4 minutes after fertilization. Note eggs broken into many small pieces and compare with control unfertilized eggs of Photograph 2.

PHOTOGRAPH 4. Eggs centrifuged 12 minutes after fertilization for 8 minutes; fertilization membranes previously removed, except for a few eggs which may be observed still nearly spherical. Photograph taken while rotating in centrifuge-microscope.

PHOTOGRAPHS 5 and 6. Retraction of eggs with slowing down of centrifuge; eggs centrifuged 10 minutes after fertilization; Photograph 5 taken 6 minutes later at high speed; then speed lowered and Photograph 6 taken 6 minutes later, same eggs. Note shortening and bending at bottom in Photograph 6.

PHOTOGRAPH 7. Eggs centrifuged 20 minutes after fertilization. Picture taken 12 minutes later while rotating. Elongate dumb-bells.

PHOTOGRAPH 8. Eggs cleaving while being rotated in centrifuge. Eggs (membranes not removed) rotated 3 minutes after fertilization at high speed (5000 × gravity) for 15 minutes, then low speed (2500 × gravity) for 1 hour. Photograph taken while rotating.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slowed down at the stage of the long streamers, these contract and bend at the bottom, being evidently quite elastic (Photographs 5 and 6, same eggs). On increasing the speed, they again elongate slightly. If the centrifugal force is removed during the streamer stage, the greatly stretched eggs may contract into spheres or they may break in one or many places and these pieces become spherical. The formation of long streamers cannot be connected with the ectoplasmic layer since this is thrown off to the centrifugal pole as a crescent, easily seen on eggs with broken fertilization membranes—a phenomenon to be described in detail in a later paper.

After about 20 minutes after fertilization, during the streak stage, the eggs when centrifuged form shorter streamers or become elongate dumb-bells (Photograph 7) and break apart less and less readily as the interval after insemination increases. In some batches of eggs, the fertilized eggs break apart at all stages (provided the fertilization membranes have been removed) more readily than the unfertilized. In other batches they break apart less readily in later stages, or do not elongate much or break apart at all with the same centrifugal force. The stage at which the fertilized eggs break less rapidly than the unfertilized differs in different batches. This resistance to breaking can be overcome by keeping the eggs after removal of the membranes in calcium-free sea water. They then elongate when centrifuged and break apart readily at any stage up to cleavage. No difference in viscosity could be detected in (unfertilized) eggs kept in artificial sea water *without* calcium (brought to the same pH as sea water by the addition of sodium bicarbonate) and those kept in similar artificial sea water *with* calcium; a comparison was made of the rate of stratification in these solutions with the double-head centrifuge-microscope. The effect of the calcium-free sea water must therefore be a surface effect, as might be expected from its well-known influence on the ectoplasmic layer, causing its disintegration and the falling apart of blastomeres (Herbst, 1900).

The general striking differences in behavior of the fertilized (in

PLATE II

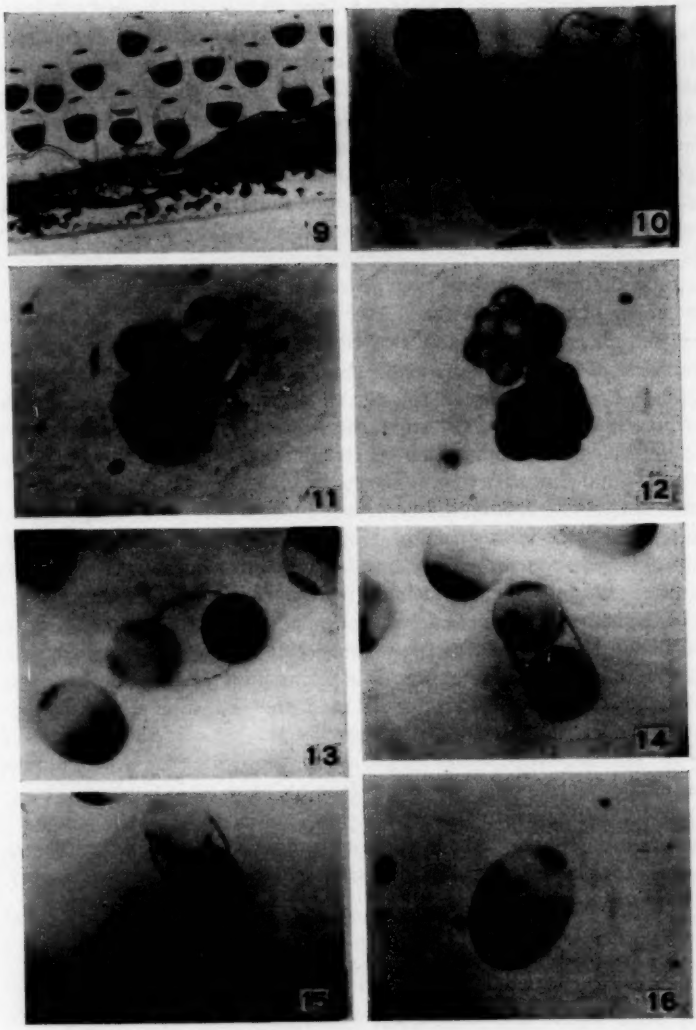
PHOTOGRAPH 9. Eggs centrifuged slowly (2500 × gravity) while fertilization membrane was being formed; two spheres inside membrane (lower left center egg). Photograph taken while rotating in centrifuge-microscope.

PHOTOGRAPH 10. Same removed from centrifuge-microscope. White sphere of upper egg has cleaved.

PHOTOGRAPH 11. Same. Development of white sphere, no development of red.

PHOTOGRAPH 12. Same. Development of both spheres.

PHOTOGRAPHS 13–16 (same egg). Same. Fusion of two spheres at first (Photograph 13) well separated within fertilization membrane.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various stages) and unfertilized eggs of *Arbacia* must be due primarily to surface changes. The more rapid breaking of the just fertilized eggs when centrifuged in spite of increased viscosity must be due to a decrease in surface forces. Cole and Michaelis (1932) have measured the surface forces of unfertilized and fertilized *Arbacia* eggs, but not during the period soon after fertilization when the breaking is most rapid; they found in fertilized eggs without fertilization membranes that there was no increase in surface forces until shortly before cleavage—a period when the eggs have become more difficult to break apart. At this stage it is possible that the great increase in viscosity may also be involved in the more difficult pulling apart. The great elasticity of the eggs at the streamer stage must also be connected with surface changes. Surface changes upon fertilization are indicated in the normal egg by the formation of the fertilization membrane and of the ectoplasmic layer, and the change in position of the pigment granules.

It is possible to break the egg apart into two spheres inside the fertilization membrane (Photographs 9, 10, 13); this is done by centrifuging the eggs immediately after fertilization but more slowly, with a force of 2500 times gravity. The fertilization membrane is frequently much stretched—from a diameter of 82 μ to a length of 128 μ. If the centrifugal force is now removed, either (1) the stretched membrane contracts, and the two spheres are brought into contact and gradually coalesce (Photographs 13–16, showing stages in complete coalescence) or (2) the two spheres remain separate and develop separately (Photographs 10–12). Since the sperm may enter the egg at any point, it may be in either sphere at the time of breaking apart. If the sperm is in the white sphere, it cleaves, and the red sphere, lacking any nucleus, does not develop (Photographs 10, 11). If the sperm is in the red sphere, both the half eggs may develop, one (the white) having the female nucleus only, the other (the red) the male nucleus (Photograph 12). The white sphere, however, sometimes does not develop when the red sphere does; in this case presumably artificial parthenogenesis is not initiated in the white sphere by the sperm before its segregation in the red sphere.

Parthenogenetic Eggs

Having found that fertilized and unfertilized eggs react quite differently to centrifugal force, observations were made on parthenogenetic eggs. Unfertilized eggs were started to develop parthenogenetically by treating them for a few seconds with distilled water; by this method, they develop only up to first cleavage. When placed immediately

after treatment in the centrifuge slide, they broke apart very readily at high speed. With low speed, acting while the (fertilization) membrane was being raised, the membrane was observed to be stretched and the egg broke into two spheres within the membrane; these two spheres sometimes remained separate and sometimes coalesced on retraction of the membrane when removed from the centrifuge. If the membranes were removed by violent shaking as soon as formed, the parthenogenetic eggs behaved exactly like the fertilized eggs, forming long streamers from five to twenty minutes after treatment, and then becoming more resistant to elongation and breaking. This could be overcome by keeping the eggs previously in calcium-free sea water. Parthenogenetic eggs act, therefore, in every way and at every stage up to the first cleavage exactly like fertilized eggs.

Cleavage during Rotation

When fertilized eggs are put in the centrifuge-microscope 45 minutes after fertilization and centrifuged continuously, cleavage comes in, even with a centrifugal force of 5000 times gravity, about 20 minutes after the uncentrifuged control. Another set of eggs was rotated at high speed (5000 times gravity) for 15 minutes immediately after fertilization, and then at a lower speed (about 2500 times gravity) for an hour, when cleavage took place while rotating (Photograph 8). In all cases observed, the cleavage plane came through the oil cap and divided the egg along its long axis. It is an interesting fact that an egg can pass through all the complicated processes connected with fertilization and cleavage while being rotated at high speed (5000 times a minute).

Summary

1. *Arbacia* eggs centrifuged immediately (30–90 seconds) after fertilization become more elongate dumb-bells and break into halves and quarters more readily (most batches) than unfertilized eggs.

2. If centrifuged one and one-half to five minutes after fertilization, they break still more readily into many small pieces.

3. If centrifuged five to twenty minutes after fertilization (fertilization membranes previously removed), they form long streamers which break along their course. After twenty minutes, they elongate and break less and less readily. Treatment with calcium-free sea water makes them break more readily.

4. Some batches of eggs break more readily at every stage after fertilization than unfertilized eggs. Other batches break more readily only up to a certain stage, then less readily, the stage varying with different batches.

5. Fertilized eggs at all stages stratify less readily (that is, are more viscous) than unfertilized. The greater ease of breaking must therefore be due primarily to surface changes.

6. If centrifuged slowly while the fertilization membrane is being formed, this may be stretched and the egg pulled apart inside the membrane. The two parts may develop separately, either one or both according to the location of the sperm; or they may fuse together.

7. Parthenogenetic eggs at all stages up to cleavage act exactly like fertilized eggs.

8. Eggs may cleave even while being rotated at high speed (5000 times gravity); the cleavage plane passes through the oil cap and elongated axis of the cell.

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ARSENIC IN SEA WATER

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Interest in the occurrence and quantity of arsenic in sea water centers chiefly around its relation to phosphorus: first, the possibility of its utilization by diatoms or other organisms, in place of or in addition to phosphorus; and second, the possible interference of arsenate in the regular routine determination of phosphate so frequently carried out upon sea water samples.

Arsenic has long been known to occur in the sea. Quantitative determinations by Gautier (1), and others (2), using laborious methods, indicated its presence in amounts of the order of 10–100 milligrams per cubic meter.

Gautier found that in surface water (from near the coast of Brittany) containing 10 mg./m.^{3} of total arsenic, 90 per cent was present in inorganic form. At one deep-sea station he found 25 mg./m.^{3} of As at a depth of 10 meters, 10 mg. at 1335 m., and 80 mg. at 5943 m. He was inclined to attribute the high value at the bottom to volcanic influences and the moderately high value in the upper level to some factor associated with diatom growth. Others, following these limited results, have regularly assumed an increase of arsenic with depth.

Chapman (3), while studying the arsenic content of shell-fish and other marine organisms, made sixteen determinations on sea water samples from near the Nore Lightship. He reports 0.14 to 1.0 parts per million, with an average of 0.33; *i.e.*, more than 300 mg./m.^{3}!

Atkins and Wilson (4), in a study of the methods for the determination of phosphate, silicate, and arsenic, have shown good reason for assuming that while arsenate interferes in the colorimetric estimation of phosphate by producing a similar color, arsenite is without effect; furthermore, that since total As is frequently in larger amounts than the colorimetric method indicates phosphate, most of the arsenic in sea water must be present in some other form, presumably arsenite.

It has been reported, on the basis of culture experiments, that arsenate can substitute for phosphate, to a certain extent, as a requirement for the growth of diatoms, but it is unknown to what extent this actually takes place in the sea.

[1] Contribution No. 29.

Data on the total arsenic content of sea water have been very scarce, and since no application seems to have been made of the Gutzeit method, the superiority of which over the older Marsh method has been frequently demonstrated, the present authors undertook to apply the more recent procedure to a number of sea water samples.

These were obtained during the summer of 1932 in the course of the regular collecting cruises of the vessels, "Atlantis" and "Asterias," of the Woods Hole Oceanographic Institution, from a number of inshore stations in the Gulf of Maine, to the south of Martha's Vineyard, off the entrance to New York Harbor, etc. In addition, two deep-sea stations are listed in the tables, No. 1349, at 38° 58′ N., 64° 30′ W., and No. 1370, at 35° 55′ N., 72° 46′ W.

TABLE I

Data on Concentration of Arsenic in Sea Water Taken at Different Depths at Various Stations

Gulf of Maine

Station	Depth	As	Station	Depth	As	Station	Depth	As
	m.	mg./m.³		m.	mg./m.³		m.	mg./m.³
P-1	0	7	H-8	0	17	1333	0	15
	60	15		10	22		9	15
F-15	0	10		20	12		17	22
	10	17		30	13		26	13
	50	21		50	10		35	16
	75	15		75	15		44	16
	100	10		100	17		52	13
G-12	0	9		160	18		65	16
	10	17	1329	0	9		87	12
	30	11		10	11		186	16
	50	10		20	12	1335	0	9
	75	15		30	10		10	11
	105	6		40	29		20	10
G-11	0	9		50	17		30	10
	10	10		60	14		40	7
	20	14		75	11		50	10
	30	12		100	17		60	10
	50	24		240	20	1337	0	15
	75	13	1331	0	17		10	12
	110	10		10	18		20	10
H-3	0	17		19	20		30	10
	10	16		29	15		40	13
	20	16		39	15		50	10
	30	14		48	11		60	11
	50	16		58	16			
	75	(28)		72	18			
	100	10		97	18			
	115	13		194	12			

TABLE I—*Continued*

N.E. of Cape Cod		
Station	Depth	As
	m.	mg./m.³
B′-5	10	20
	20	20
	30	25
B′-20	0	18
	20	22
	100	20
	243	25

Off Entrance of N. Y. Harbor		
Station	Depth	As
	m.	mg./m.³
1297	0	9
	60	22
1298	0	16
	70	26

South of Martha's Vineyard		
Station	Depth	As
	m.	mg./m.³
A-4	0	10
	10	17
	15	11
A-8	0	14
	10	14
	20	17
	26	8

South of Martha's Vineyard (cont.)		
Station	Depth	As
	m.	mg./m.³
A-25	0	10
	10	11
	20	21
	30	7
	40	10
	50	10
A-28	0	11
	10	21
	20	14
	30	21
	40	(9)
	50	(27)
	57	10

Long. 38° 58′ N. Lat. 64° 30′ W.		
Station	Depth	As
	m.	mg./m.³
1349	0	28
	200	17
	300	12
	400	20
	600	12
	800	16
	1000	17

Long. 38° 58′ N. Lat. 64° 30′ W. (cont.)		
Station	Depth	As
	m.	mg./m.³
1349	1200	18
	1600	20
	2000	15
	2500	16
	3000	20
	4000	15

Long. 35° 55′ N. Lat. 72° 46′ W.		
Station	Depth	As
	m.	mg./m.³
1370	0	54
	50	22
	100	21
	200	20
	400	20
	600	22
	800	(31)
	1200	16
	1600	17
	2000	16
	2500	15
	3000	13

The Gutzeit method (5), as now commonly employed in the analysis of foods etc., was used without serious modification, concentrating 100 cc. of the water to small volume before use. The length of the stain on the $HgBr_2$-paper was compared for each sample with those resulting from standard solutions of arsenic. The results, with descriptions of the stations and the depths in meters from which the samples were taken, are given in the tables. Figures indicate milligrams of arsenic per cubic meter of water, *i.e.*, parts per billion.

The results from Station K-2, in Buzzards Bay, represent a series of samples taken successively from the same station over a period of several hours and afford, perhaps, a fair indication of the accuracy of the method. Only one variation of more than 6 mg./m.³ is found at

corresponding depths, and this range must include not only experimental errors but also any actual variation in the water during the time.

In the case of the shallow stations there seems to be a distinct tendency for an increase in arsenic a short distance below the surface, usually with a decrease again at greater depths. This tendency is far from consistent, however, which is to be expected if it is associated with the variable conditions of the phytoplankton, or diatoms, from one station to another.

The two deep stations present a different problem, with their unusually high surface values, which may, perhaps, be purely accidental. But, more important, there is no regular or significant increase of arsenic with depth. Contrasting this with the case of phosphorus, which, at such a station, would show a ten-fold increase, at least, between the surface and bottom, shows that the two elements are utilized in very different manner.

TABLE II

Data on Concentration of Arsenic at Station K-2 in Buzzards Bay at Different Times of Day

Depth	Time				
	6:25 A.M.	9:31 A.M.	12:37 P.M.	3:43 P.M.	6:49 P.M.
m.	*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}	*mg./m.*^{3}
0	15	16	11	13	13
5	15	14	14	16	14
10	10	16	12	15	20
15	13	17	15	11	

Out of 155 determinations made, 140, or 90 per cent, fall between 9 and 22 mg./m.^{3} of As. This small range may, we think, be taken as the normal. Only seven samples were found with more than 25 mg. The average and median of all determinations, irrespective of depth or locality, were each 15 mg./m.^{3}.

The data yield two important general results:

1. Failure to bear out the previous findings of Gautier, who reported a considerable increase in As concentration with depth. On the contrary, although a small amount of the As may be removed from the water at certain stations by diatoms etc., this process is not nearly so important or general as in the case of phosphorus.

2. Corroboration of the assumption of Atkins and Wilson that the greater portion of the As in sea water is present in the form of arsenite.

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DIURNAL MIGRATION OF PLANKTON IN THE GULF OF MAINE AND ITS CORRELATION WITH CHANGES IN SUBMARINE IRRADIATION

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The investigations of Russell (1926, 1927, 1931), Kikuchi (1930), Worthington (1931), and Southern and Gardiner (1932) reveal the magnitude and the importance of the diurnal migrations of zoöplankton. The observations indicate that light is probably the most important factor controlling these movements, although it is uncertain in just what way the organisms respond to changes in the intensity of light. There is likewise considerable doubt in regard to the parts played by other factors. The present investigation was undertaken to study in more accurate detail the migrations of plankton organisms —particularly copepods—and to test the theories regarding the actual rôle of light.

In previous investigations the intensity of the light in the water was not measured at the same time that the plankton hauls were made.[2] Usually the submarine irradiation was assumed to be proportional to the light present at the surface as indicated by the time of day and the condition of the weather. Russell (1931) has estimated values for the irradiation at various depths on the basis of measurements made on other occasions (Poole and Atkins, 1929). In the present investigation it was therefore proposed to record the irradiation on deck throughout daylight hours when plankton hauls were in progress and to measure the percentage of surface light penetrating to various depths in the water at suitable periods during the day. The intensity of light to which the organisms are subjected at different levels would thus be precisely known.

To improve the accuracy of the determination of the vertical distribution of the animals, five nets were constructed which could be towed simultaneously at different depths and opened and closed in position. I am indebted to Mr. C. O'D. Iselin of the Woods Hole

[1] Contribution No. 33.

[2] Russell, in collaboration with Atkins, has recently made such observations, the results of which are as yet unreported (see Atkins and Poole, 1933).

Oceanographic Institution for the main ideas involved in the construction of these nets and for the actual design of the fittings. By using these at intervals during the day and night, we hoped to obtain significant information in regard to the vertical migrations taking place.

The observations on the diurnal migration of plankton reported in this paper were made from the "Atlantis," the research vessel of the Woods Hole Oceanographic Institution, at Station 1053 (in August, 1931) and at Stations 1285, 1286, and 1287 (in July, 1932)—all in the Gulf of Maine. The details of the measurement of the penetration of daylight are presented in a separate paper (Clarke, 1933).

Method of Making Hauls

The five closing nets were clamped at intervals on the main dredging cable and towed horizontally through the water, thus sampling the plankton at five different depths in one operation. The original nets were made of silk but these were abandoned because they were too easily injured by washing against the cable—a circumstance unavoidable in the method used. The nets employed in 1932 were made of heavy cotton "scrim" (10 strands/cm.) and proved very satisfactory. They are 3 meters long, 75 cm. in diameter at the mouth, 11 cm. in diameter at the bottom, and provided with a pursing rope 1 meter from the mouth.

The canvas sleeve at the bottom of each net fits snugly over the rim of a light brass bucket (11 cm. in diameter and 17.5 cm. deep), similar to that described by Kemp and Hardy (1929). A brass band is slipped over the canvas and clamped tight by means of a bolt with winged nut. The bucket is thus secured firmly to the net and can be attached or removed quickly. An opening on one side covered with netting of monel metal (40 strands/cm.) allows about two-thirds of the water in the bucket to drain off so that the catch can be poured directly into a 1/2 liter bottle without further straining.

The main dredging cable (1.27 cm. in diameter) to which the five nets are attached, travels from the winch forward along the deck and then outboard to the end of a boom located amidships. The cable is kept as nearly vertical as possible by a stream-lined weight (680 kilograms in air) fastened to the lower end. During this investigation the angle which the cable made with the vertical never averaged more than 23° for each towing period. The work of attaching the nets to the cable is performed at the end of a platform rigged out from the side of the ship.

Each net is laced to its frame, consisting of a brass hoop, and this is attached directly to the cable by means of clamps, no bridle being

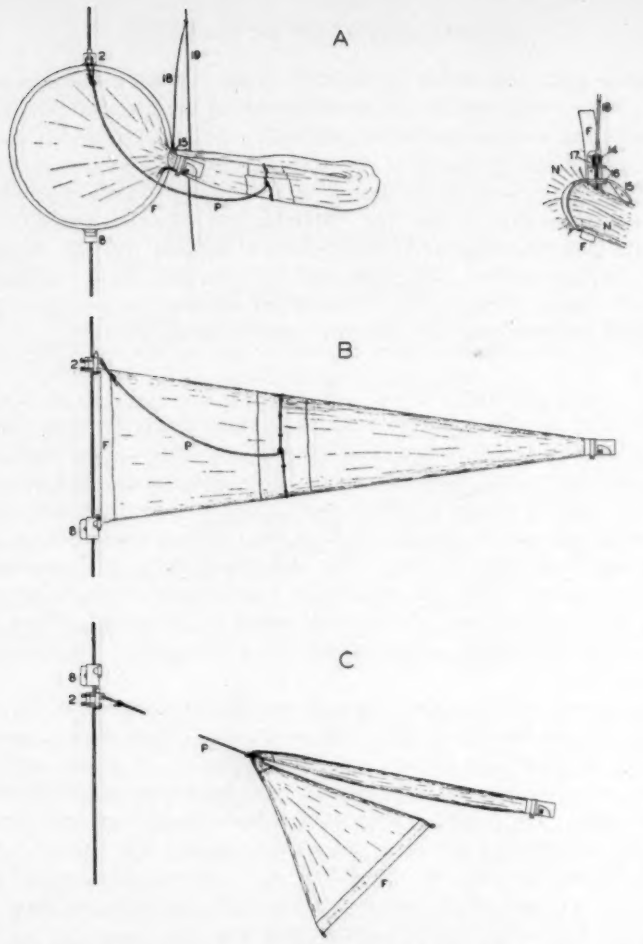

FIG. 1. Diagrams to show the method of using the nets.

A. Net closed and arranged for lowering preparatory to making haul. Net frame moves edgewise through the water.

Inset: Enlargement of edge of net frame showing in detail the strap and pin device.

B. Net open and fishing. The opening is unobstructed except for the single cable passing across it.

C. Net closed and ready to be hoisted aboard. The net frame has been cast off and the net is drawn shut on the pursing rope. Note the "messenger" clamp (8), which has dropped from the net above on to the trigger of the upper clamp (2), thus releasing the closing mechanism.

14. Pin.
15. Strap.
16. Brackets.
17. Thread stopper.
18. Cord attaching pin to (19).
19. Hand-line to which all the pins are attached.

F. Frame.
N. Netting.
P. Pursing rope.

used (see Fig. 1, *B*). The top clamp is composed of two separate parts, as shown in Fig. 2, *A*. The split lug (1), which is cast to fit the lay of the cable, is first rigidly fixed in position by tightening the four screws. Since the lug is small enough to pass through the sheaves, this operation may be performed on deck and the cable then lowered away until the lug reaches a position about two meters above the water. The second part (2), of the upper clamp, is next closed around the lug as shown in *B* (Fig. 2). This fitting swivels freely around the lug. The upper edge of the net frame is provided with a flanged plate (3) which is next secured in the slot (4) of the fitting. This is accomplished by depressing against an internal spring the trigger end (5) of the U-shaped bolt (6). The latch end (7) of the bolt is thus withdrawn from the slot (4), and plate (3) is allowed to slip in. When pressure on the trigger

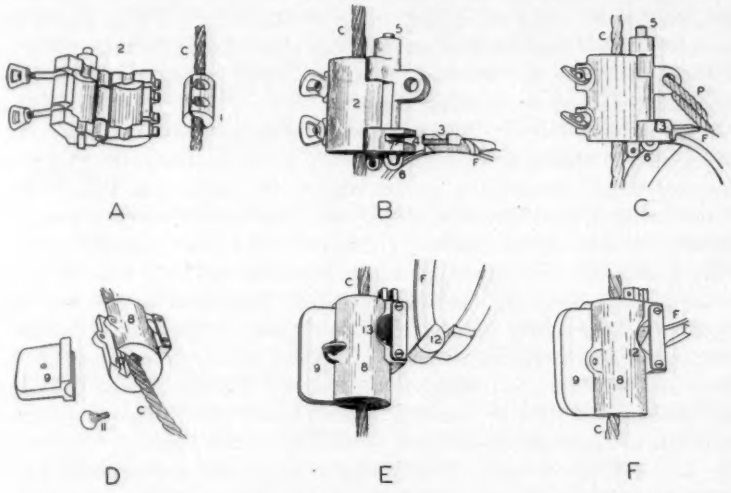

FIG. 2. Method of attaching closing net to cable. For explanation see text.

A, *B*, and *C*—upper clamp and top of net frame.
D, *E*, and *F*—lower clamp and bottom of net frame.

1. Split lug secured to cable.
2. Outside part of upper clamp which encloses (1).
3. Flanged plate at top of frame.
4. Slot into which (3) fits.
5. Trigger end of (6).
6. U-shaped bolt.
7. Latch end of (6).
8. Cylindrical part of lower clamp.
9. Key which slips into (10).
10. Groove in (8).
11. Thumbscrew.
12. Projection at bottom of frame.
13. Socket into which (12) fits.

C. Main dredging cable.
F. Net frame.
P. Pursing rope.

is released, the latch (7) snaps back, passing through a hole in plate (3) and locking it into position (*C*, Fig. 2). The pursing rope (*P*) is made fast to the eye provided in this fitting.

The next step is placing the lower clamp in position as shown in *D* (Fig. 2). The cylindrical part (8) is slipped over the cable and the key (9) is slid up into the groove (10) where it is held by the thumbscrew (11). Before this is done, however, the clamp must be attached to the lower edge of the net frame. This is provided with a projection (12) in the shape of a 3/4-cylinder. The projection can slip into or out of the similarly-shaped socket (13), which is open in front, except when the net frame and the longitudinal axis of the fitting (8) are held parallel. Accordingly, in attaching the lower clamp the fitting (8) is first tilted until the projection (12) slips into the socket (13). It is then straightened up, thus locking the projection in, and the key (9) is slid into place, thus securing clamp and frame to the cable (*F*, Fig. 2). The whole clamp is free to swivel around the cable. Since the upper fitting (2) can also turn, the net frame is free to rotate horizontally although it cannot slide up or down on the cable.

To prevent the net from fishing on the way down (and while the other nets are being attached), it is closed at the mouth by drawing the netting across the opening to one edge of the frame (see Fig. 1, *A*). The netting is held here by a strap, the lower end of which is permanently fastened to the frame. The upper end is provided with a hole (see inset, Fig. 1, *A*) through which the brass pin (14) passes, thus securing the strap (15) between the two brackets (16). A thread stopper (17) prevents the pin from coming out fortuitously. The tail of the net is doubled back and placed under the strap, because if left free, it would lash about and become fouled. The pin (14) is attached to the hand-line (19) by the short piece of cord (18). The pins of all the nets are similarly fastened in succession to this line.

The net is now ready for lowering and the ship is put under way at the lowest possible speed. As soon as the net is in the water, it is swung around by the loose netting trailing out behind. The net frame accordingly tows edgewise through the water and does not fish. Meanwhile the next lug has been attached to the cable at the proper distance inboard. This is run out to the position over the water where the next net is attached exactly as before.

When all the nets have been attached to the cable and the whole string lowered to the desired depth, the ship is accelerated to the regular towing speed (see below). The nets are then opened simultaneously by hauling on the handline (19). When all the pins are pulled free, the line with the five pins attached is taken in on deck. Instantly

the pressure of the water causes the nets to open out and to fish as shown in *B*, Fig. 1.

At the end of the towing period the nets are all closed before they are raised to the surface. This is accomplished as follows. A "messenger" (an extra lower clamp) is placed on the cable and allowed to slide down it. When this reaches the topmost net, it strikes the trigger (5). The latch end (7) of the bolt is thereby forced downward and the plate (3) is pulled free by the horizontal pressure of the water on the net. Once the frame of the net tilts backward in this way, the projection (12) slips out of its socket and the frame becomes detached from the lower clamp. Since there is now no vertical support for the lower clamp, it immediately slides down the cable and acts as the "messenger" to release the next net. In quick succession the frames of all five nets are thus cast off from the cable, leaving the nets hanging from the upper clamps by their pursing ropes only. The drag of the net through the water causes these to draw tight and to effectively close the nets as is shown in *C*, Fig. 1. Finally, the nets are raised and taken on deck in the reverse order in which they were sent down. With a little practice this whole operation, including the 10-minute tow, can be completed and the nets made ready to be sent down again in about three-quarters of an hour.

The actual depths at which the five nets were towed at the different stations are given in the tables. In general, the nets were spaced in such a way as to sample the upper 50 meters of water to the best advantage. During the middle of the day, when the plankton was expected to occur at its maximum depth, the series of hauls was repeated at a lower level, the five nets then sampling the water between 50 meters and 100 meters. On one day this was done at other hours as well. The angle which the cable made with the vertical was measured in the usual way at the beginning, middle, and end of each towing period and was found to vary ordinarily between 10° and 20°. In those few cases in which the angle during this period averaged more than 20°, the depths of all the nets were corrected by multiplying by the cosine of the average angle.

The towing period was 10 minutes in all cases. The speed of the ship was carefully determined at least three times during the tow by measuring with a stop-watch the time required for a section of the ship's deck 15.2 meters long to pass a chip of wood tossed into the water. By adjusting the speed of the ship from time to time, the average speed for each tow was kept within the limits of 1.0 to 1.8 knots. At a speed of 1.25 knots, the average for all the hauls, the nets would be drawn through 366 meters of water during the 10-minute period,

thus theoretically straining 162 cubic meters. Although the speed of towing varied slightly, it is important to note that it was necessarily the same for all five nets on the line. Differences in the number of animals caught at different depths at the same time, therefore, could not be due to variation in the towing speed.

The hours at which the series of plankton hauls were made are indicated in the tables. At Station 1053 five series were made during the morning. Further observations were unfortunately prevented by bad weather. During the 24-hour period comprising Stations 1285 and 1286, four series were made and each repeated at the lower level. At Station 1287 nineteen series (2 repeated) were made during a continuous 48-hour period. In general the series were made at more frequent intervals of time during the early morning and in the evening because the plankton animals are known to migrate most rapidly during those periods.

Method of Estimating Catches

The plankton catches were preserved in 10 per cent formalin and transported to the laboratory where the numbers of the different kinds of organisms were estimated by the following sampling method. Each catch was poured into a large cylindrical battery jar graduated in liters. Water was then added until a suitable dilution was attained, the total volume varying, according to the size of the catch, from 1 liter to 10 liters. The liquid was then thoroughly stirred by an electrically driven propeller. As soon as the plankton organisms became evenly distributed, four samples were taken with a dipper of 29-cc. capacity (consisting of a small porcelain crucible provided with a long handle).

Each sample was poured out upon a ruled counting slide (3.5 × 7.2 cm.) and placed under the binocular microscope. By the use of a mechanical stage the whole sample can be rapidly passed in review. Each individual was examined in turn and its species, sex, and age group recorded. The analysis was limited to those forms which occur in significant numbers. If the first two samples did not vary by more than 10 per cent from their average, no more samples were counted. The samples often gave counts closer than this, indicating that the method used is sufficiently accurate for the purpose (see below). If agreement was not good, the third and, if necessary, the fourth sample was examined. In the case of very large catches it was sometimes necessary to sub-sample the original samples. The same method was used but more samples were counted to insure the same degree of accuracy.

Sources of Error

One of the most serious sources of error occurring in investigations of this type is the irregularity in the horizontal distribution (*i.e.*,

"swarming") of the plankton. The present method, employing five nets towed simultaneously on a single cable, has certain advantages over previous methods in reducing the magnitude of this error. In the first place, the five hauls of a single series contain no error at all from this source when compared among themselves since they were made at the same time and place. But since the successive series of hauls are not made in exactly the same body of water (due to the drift of the ship), "swarming" would introduce an error for comparisons between the hauls in different series. However, since the nets are towed a considerable distance horizontally, this error is less than would result from the use of a single net hauled vertically (cf. Southern and Gardiner, 1932; and Worthington, 1931). This method is likewise an improvement over those in which a single horizontal net has been towed at different depths in succession. In such cases the individual hauls have been made in different bodies of water, thus introducing the possibility of an error within each series (cf. Russell, 1925).

The use of five nets at once has eliminated another error previously encountered. This was due to the relatively long interval between the times when the first haul and the last haul of a series were made and the consequent possibility that the distribution of the plankton had changed meanwhile. Furthermore, the present method is sufficiently quick to enable complete observations to be repeated at intervals of less than an hour.

In view of the precautions taken, as described above, the errors due to differences in speed and duration of towing (and hence in amount of water strained) and in the depths of the nets are probably negligible. Moreover, it is important to note that the speed and duration are of necessity the same for all the hauls in the same series. The distance between the nets on the cable remained the same, of course, throughout each towing period, and could not have varied by more than a fraction of a meter in different series.

The use of closing nets which are allowed to fish only at their proper depths increases the accuracy of the observations over that possible when open nets are used. However, since the nets are towed horizontally, the layers of water between the nets are not sampled and in the present case there was a considerable body of water beneath the lowest net the population of which was not investigated at all. (Bottom at 256 meters at Stations 1285 and 1286, 146 meters at Station 1287.) Since the investigation was undertaken primarily to test the effect of light, attention was centered on the surface layers. Even at the brightest time of day, light sufficiently intense to be measured did not penetrate to a depth greater than about 50 meters. In the case of the or-

ganisms for which we have the most accurate information (*Metridia lucens* adult female), the zone of maximum abundance did not sink below the 54-meter depth at the hour when it would be expected to occur at its lowest for the day (see Figs. 3 and 5). It seems probable that the picture of the vertical distribution of this form would not be materially altered if information on the population of the water layers between the levels of the five nets could have been obtained.

Certain other sources of error common to all similar investigations should be mentioned, the importance of which has long been in question. First of all, there is the possibility that because of clogging the nets might vary in the amount of water actually strained. No satisfactory method has yet been devised to remove this source of error. In the present work the precaution of thoroughly washing out the nets after each haul was always taken. Probably more serious is the possibility that the animals escape the net to varying degrees on different occasions. The speed of towing and the size of the mouth of the net (cf. Gardiner, 1933) are important factors in this connection, but whatever their effects may have been in the present investigation, they were the same for all the hauls. Since our nets have no bridles, the water is stirred up to a minimum extent in front of their openings. Whatever ability the plankton animals may have to see the net and to avoid it to a greater extent in bright light than in weak light or darkness would tend to cause differences in the numbers caught (1) between surface catches made during the day and those made at night and (2) between daytime catches made at the surface and those made at depths where the light is greatly reduced. This question has been thoroughly discussed by Russell (1928), Worthington (1931), and Southern and Gardiner (1932). It is useless to compare day and night totals in the present case (as previously done), since no attempt was made to investigate the bottom layers of water.

Finally, the sources of error in the estimation of the catch must be examined. The method used is comparable to those employed by Russell (1925) and Worthington (1931), which appear to be satisfactory. In an extensive investigation of this matter Gardiner (1931) found that the enumeration error when using a similar method (the Hensen Stempel-pipette) was insufficient to account for the variation of the catch. The error resulting from sampling is undoubtedly large, but in view of the huge variation in numbers of animals caught it is probably *relatively* small, if not negligible.

It seems permissible to conclude that the methods used in this investigation are at least as accurate as those previously employed and that the results may be relied upon. The modifications which have

been introduced—particularly the use of five simultaneous closing nets —allow special dependence to be placed upon the differences found among the hauls within a single series.

Observations

General Vertical Distribution

The following three species of copepods comprise the bulk of the catches made: *Metridia lucens* (Boeck), *Centropages typicus* (Krøyer), and *Calanus finmarchicus* (Gunner). In most of the hauls made at Stations 1285 and 1286 and in a few of those at Station 1287, quite large quantities of siphonophore fragments were taken as well. A general view of the numbers of each species of copepod present and of the relative depth at which each lives may be obtained from Fig. 3.

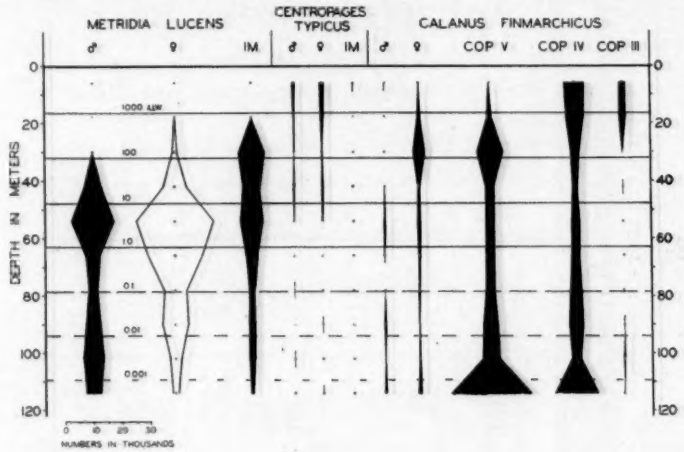

FIG. 3. General view of the vertical distribution and the relative numbers of the three species of copepods studied. The diagram for the adult females of *Metridia* is plotted on one-half the scale of the others. The depth at which light intensities of 1000 microwatts, 100 microwatts, etc., occurred are indicated by the horizontal lines. (One microwatt may be taken as approximately equal to three meter-candles as used by Poole and Atkins, 1929.) The upper five hauls (A244 to A248) were made at 1439 o'clock, the lower five (Nos. A249 to A253) at 1515 o'clock on July 11, 1932, at Station 1287.

The diagrams, constructed in the usual way (cf. Russell, 1925), represent the copepod population down to 114 meters at Station 1287 early in the afternoon of July 11, 1932. This particular series has been selected as being the most complete and representative. The numerical data for all the series are presented in Tables I, II, and III.

TABLE I

Station 1053, August 24, 1931, Lat. 43°27′N., Long. 67°39′W. Sea rough, sky overcast. Sunrise at 0511. Numbers of copepods in thousands. x = less than 500.

Haul No.	Depth	Local Sun Time †	Irradiation at Surface	Irradiation at Haul Depth	Irradiation 30 min. before	Centropages typicus			Calanus finmarchicus				
						Adult ♂	Adult ♀	Immature	Adult ♂	Adult ♀	Cop. V	Cop. IV	Cop. III
	meters		*microwatts*	*microwatts*	*per cent*								
A55	4.5	0456	19	9.3	No light	282	223	648	0	0	0	0	0
56	14.	"	"	2.7	" "	12	26	4	0	x	x	1	2
57	23.	"	"	1.0	" "	2	9	1	x	11	92	52	27
58	32.	"	"	—	" "	x	2	1	x	15	88	34	3
59	46.	"	"	—	" "	1	2	1	x	6	15	2	0
60	4.5	0602	970	480.	49	16	25	308	0	0	0	0	0
61	14.	"	"	140.	"	103	107	150	0	0	0	0	0
62	23.	"	"	50.	"	1	x	x	x	18	77	53	36
63	32.	"	"	9.1	"	x	x	0	1	13	79	61	11
64	46.	"	"	2.0	"	x	1	x	0	2	11	4	0
65	4.5	0656	1930	950.	57	4	10	153	0	0	0	0	0
66	14.	"	"	270.	"	94	77	69	x	x	x	x	x
67	23.	"	"	100.	"	x	1	x	0	2	26	28	12
68	32.	"	"	18.	"	x	x	x	x	3	32	27	6
69	46.	"	"	4.1	"	x	1	0	x	2	21	13	x

TABLE I—*Continued*

Haul No.	Depth	Local Sun Time †	Irradiation at Surface	Irradiation at Haul Depth	Irradiation 30 min. before	Centropages typicus			Calanus finmarchicus				
						Adult ♂	Adult ♀	Immature	Adult ♂	Adult ♀	Cop. V	Cop. IV	Cop. III
	meters		*microwatts*	*microwatts*	*per cent*								
70	4.5	0807	5000	2450.	64	9	11	296	0	0	0	0	0
71	14.	"	"	700.	"	1	1	35	0	0	0	0	0
72	23.	"	"	260.	"	26	21	84	0	0	0	0	0
73	32.	"	"	47.	"	2	4	1	0	*x*	*x*	*x*	*x*
74	46.	"	"	10.5	"	*x*	*x*	*x*	*x*	*x*	*x*	1	*x*
75	4.5	1227	7100	3500.	150	9	14	360	0	0	*x*	0	0
76	14.	"	"	990.	"	7	12	115	0	0	0	0	0
77	23.	"	"	370.	"	194	220	400	0	0	0	*x*	26
78	32.	"	"	66.	"	2	3	2	0	*x*	11	25	23
79	46.	"	"	15.	"	*x*	1	*x*	0	2	5	5	1
80	64.	1330	7500	3.4	76	0	*x*	*x*	0	2	8	5	*x*
81	83	"	"	—	"	1*	2*	1*	*x*	1	3	1	*x*
82	101	"	"	—	"	*x**	*x**	*x**	*x*	*x*	2	1	*x*
83	120	"	"	—	"	*x**	*x**	1*	0	*x*	2	1	0

* Mostly cast shells.

† 24-hour scale. The time indicated is the middle of the 10-minute towing period.

TABLE II

Hauls A145–A174, Station 1285, July 9, 1932, Lat. 42°34′N., Long. 69°37′W. Sea, slight; sky, clear. Sunrise 0431. Sunset 1939.
Hauls A175–A183, Station 1286, July 10, 1932, Lat. 42°30′N., Long. 69°37′W. Sea, slight; sky, few clouds. Sunrise 0431. Sunset 1938.

Numbers of copepods in hundreds. x = less than 50.

Haul No.	Depth	Local sun time	Irrad. at surface	Irrad. at haul depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus				Metridia lucens						
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Ad. ♂	Ad. ♀	Cop. V ♂	Cop. V ♀	Cop. IV ♂	Cop. IV ♀	Cop. III
	m.		*μ W*	*μ W*	*per cent*														
A145	5	1119	14,900	6900.	100	*x*	5	*x*	0	9	9	9	0	0	*x*	0	0	0	0
146	15	"	"	1500.	"	0	0	0	0	2	13	6	0	0	2	2	0	0	0
147	25	"	"	330.	"	0	0	0	*x*	7	15	5	0	0	*x*	*x*	0	0	0
148	35	"	"	46.	"	0	0	0	0	2	20	14	*x*	*x*	3	10	*x*	*x*	0
149	45	"	"	13.	"	0	0	0	0	4	29	19	*x*	*x*	1	4	*x*	1	*x*
150	55	1230	16,200	3.7	90	0	0	0	*x*	2	18	19	1	*x*	3	6	*x*	1	0
151	65	"	"	—	"	0	0	0	0	3	23	26	1	1	4	14	*x*	2	0
152	75	"	"	—	"	0	0	0	0	3	43	65	3	1	4	14	*x*	1	0
153	85	"	"	—	"	0	0	0	0	7	58	84	8	2	7	26	3	6	0
154	95	"	"	—	"	0	0	0	0	3	96	119	9	3	5	22	0	*x*	0
155	5	1812	2,700	950.	180	4	15	1	0	2	2	3	0	0	*x*	0	0	0	0
156	15	"	"	240.	"	1	5	*x*	0	2	4	2	0	0	*x*	*x*	0	0	0
157	25	"	"	110.	"	*x*	3	0	0	2	10	6	*x*	*x*	2	6	0	0	0
158	35	"	"	12.	"	*x*	1	0	0	*x*	1	3	*x*	*x*	1	4	0	*x*	0
159	45	"	"	2.7	"	*x*	*x*	0	0	*x*	5	8	*x*	*x*	2	5	*x*	*x*	0
160	55	1851	1,500	—	"	*x*	*x*	0	0	2	35	34	6	7	1	12	0	*x*	0
161	65	"	"	—	"	0	*x*	0	0	4	51	58	7	5	*x*	5	0	*x*	0
162	75	"	"	—	"	0	0	0	*x*	3	53	64	6	6	2	6	0	0	0
163	85	"	"	—	"	0	0	0	0	3	36	43	3	1	1	3	0	0	0
164	95	"	"	—	"	2	4	0	0	6	48	37	1	7	3	6	0	0	0

TABLE II—*Continued*

Haul No.	Depth	Local sun time	Irrad. at surface	Irrad. at haul depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus				Metridia lucens						
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Ad. ♂	Ad. ♀	Cop. V ♂	Cop. V ♀	Cop. IV ♂	Cop. IV ♀	Cop. III
	m.		*μ W*	*μ W*	*per cent*														
165	5	2123	No light	No light	No light	3	17	1	0	2	5	3	0	2	*x*	3	0	0	0
166	15	"	" "	" "	" "	*x*	2	*x*	0	1	2	*x*	0	1	*x*	4	0	0	0
167	25	"	" "	" "	" "	*x*	0	0	0	3	26	14	1	16	1	8	0	0	0
168	35	"	" "	" "	" "	0	*x*	0	0	3	30	41	2	11	1	6	0	*x*	0
169	45	"	" "	" "	" "	0	*x*	0	*x*	3	29	21	*x*	18	0	6	0	*x*	0
170	55	2214	" "	" "	" "	0	0	0	0	6	42	44	4	72	2	7	0	0	0
171	65	"	" "	" "	" "	0	0	0	0	5	53	58	3	76	3	14	*x*	1	0
172	75	"	" "	" "	" "	0	0	0	0	5	53	68	5	75	5	13	0	*x*	0
173	85	"	" "	" "	" "	0	*x*	0	0	3	40	54	7	58	3	15	0	*x*	0
174	95	"	" "	" "	" "	0	0	0	1	4	40	49	4	76	2	16	*x*	*x*	0
175	5	0552	2,180	1200.	48	8	28	1	0	2	3	3	0	*x*	0	*x*	0	0	0
176	15	"	"	350.	"	*x*	*x*	0	*x*	6	11	7	*x*	*x*	4	8	0	0	0
177	25	"	"	100.	"	*x*	0	0	0	3	21	29	3	1	3	8	*x*	*x*	0
178	35	"	"	21.	"	*x*	*x*	0	0	2	12	25	2	2	2	7	0	*x*	0
179	45	"	"	4.4	"	*x*	*x*	0	0	2	19	43	2	3	1	5	0	*x*	0
180	55	0631	3,950	0.5	63	0	0	0	0	5	41	73	6	1	3	9	0	0	0
181	65	"	"	—	"	0	0	0	0	9	41	85	9	1	2	10	0	0	0
182	75	"	"	—	"	0	0	0	*x*	2	11	19	5	*x*	1	6	0	*x*	0
183	85	"	"	—	"	0	0	0	0	3	24	37	6	1	3	7	0	0	0

TABLE III

Station 1287. Lat. 43°04′N., Long. 69°24′W.

		Sea	Sky	Sunrise	Sunset
Hauls A184–A218	July 10, 1932	Smooth	Clear	0431	1938
Hauls A219–A273	July 11, 1932	Moderate	Overcast, becoming clear	0432	1938
Hauls A274–A293	July 12, 1932	Slight	Clear	0433	1939

Number of copepods in thousands. *x* = less than 500.

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
A184	6	1418	15,800	7,400.	102	4	11	1	*x*	1	1	4	1	0	0	0
185	18	"	"	1,100.	"	3	4	0	0	1	1	4	4	0	0	0
186	30	"	"	170.	"	0	*x*	*x*	0	2	4	2	*x*	0	0	*x*
187	42	"	"	19.	"	0	0	0	*x*	1	3	2	0	9	50	12
188	54	"	"	—	"	0	0	0	*x*	1	5	1	0	13	141	25
189	66	1505	13,400	—	108	0	0	0	0	1	4	1	0	3	64	3
190	78	"	"	—	"	*x*	*x*	0	0	*x*	4	2	0	3	10	3
191	(Lost)															
192	102	"	"	—	"	0	0	0	0	0	6	5	*x*	0	1	2
193	114	"	"	—	"	*x*	*x*	*x*	0	0	11	5	0	*x*	2	1
199	6	1821	2,050	960.	147	4	7	*x*	0	1	1	2	2	0	0	*x*
200	18	"	"	140.	"	*x*	0	0	0	2	9	5	3	0	0	2
201	30	"	"	22.	"	0	0	0	0	*x*	4	1	*x*	0	1	1
202	42	"	"	2.5	"	*x*	0	0	*x*	1	2	2	*x*	8	45	21
203	54	"	"	—	"	0	0	0	*x*	1	2	1	0	24	89	18
204	6	1854	1,190	560.	173	2	6	0	0	*x*	1	3	1	0	*x*	0
205	18	"	"	80.	"	0	0	0	0	2	6	5	2	0	0	1
206	30	"	"	13.	"	0	0	0	0	1	9	3	0	0	4	2
207	42	"	"	1.4	"	0	0	0	0	0	3	2	0	10	78	22
208	54	"	"	—	"	0	0	0	0	1	3	2	*x*	6	48	13

TABLE III—*Continued*

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
209	6	1952	16	8.	267	5	7	2	0	1	1	11	5	0	*x*	1
210	18	"	"	1.1	"	0	*x*	*x*	*x*	3	2	1	1	0	3	1
211	30	"	"	—	"	0	0	0	0	1	12	2	*x*	0	34	3
212	42	"	"	—	"	0	0	0	0	1	8	3	0	3	91	12
213	54	"	"	—	"	*x*	*x*	0	0	1	2	1	0	13	23	14
214	6	2107	0	0	No light	4	7	1	0	1	1	6	3	0	6	2
215	18	"	"	0	" "	0	0	0	0	7	11	12	4	*x*	46	7
216	30	"	"	0	" "	0	0	0	0	5	21	5	0	0	48	5
217	42	"	"	0	" "	0	0	0	*x*	1	2	1	0	7	12	6
218	54	"	"	0	" "	0	*x*	0	*x*	*x*	4	3	0	12	12	9
219	6	0356	0	0	No light	6	5	1	*x*	2	1	4	1	0	3	1
220	18	"	"	0	" "	0	*x*	0	0	1	5	3	0	*x*	25	6
221	30	"	"	0	" "	0	0	0	0	0	2	1	0	*x*	9	3
222	42	"	"	0	" "	0	0	0	0	1	2	1	0	1	6	4
223	54	"	"	0	" "	*x*	0	0	*x*	1	1	1	0	1	4	3
224	6	0441	114	33.	No light	3	3	2	0	1	1	5	1	0	0	1
225	18	"	"	7.1	" "	*x*	1	0	*x*	2	3	3	*x*	*x*	7	1
226	30	"	"	1.0	" "	0	0	0	0	2	15	3	0	0	32	10
227	42	"	"	—	" "	0	0	0	0	3	8	2	0	2	42	14
228	54	"	"	—	" "	0	0	0	*x*	2	10	2	*x*	6	57	14
229	6	0540	800	230.	24	0	2	1	*x*	1	1	4	1	*x*	1	1
230	18	"	"	50.	"	0	*x*	0	*x*	2	1	2	1	0	7	1
231	30	"	"	7.4	"	0	0	0	0	1	5	3	0	0	12	3
232	42	"	"	1.4	"	0	0	0	0	1	4	1	0	1	17	7
233	54	"	"	—	"	0	*x*	*x*	*x*	1	2	1	0	3	9	5

TABLE III—*Continued*

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
234	6	0742	5,360	1,600.	49	2	2	x	x	x	1	4	2	0	0	0
235	18	"	"	330.	"	0	x	0	x	4	6	5	1	0	1	2
236	30	"	"	49.	"	0	0	0	0	5	12	5	0	x	39	7
237	42	"	"	9.1	"	x	0	0	0	3	5	2	0	2	51	7
238	54	"	"	—	"	0	0	0	0	2	5	2	0	2	68	10
239	6	1109	17,100	6,000.	Variable	1	1	0	0	0	x	3	2	0	x	x
240	18	"	"	1,800.	"	1	1	0	x	1	1	2	x	0	x	x
241	30	"	"	190.	"	x	0	0	x	7	33	9	0	0	1	5
242	42	"	"	38.	"	0	x	0	x	3	7	3	x	8	8	12
243	54	"	"	16.	"	0	0	0	x	2	5	3	0	6	40	6
244	6	1439	13,900	4,900.	95	1	1	x	x	x	1	7	2	0	0	0
245	18	"	"	1,500.	"	x	1	0	0	1	2	6	2	0	x	x
246	30	"	"	150.	"	0	0	0	0	4	9	3	0	0	3	9
247	42	"	"	31.	"	x	x	0	0	1	3	2	x	6	15	6
248	54	"	"	13.	"	0	0	0	x	1	3	3	0	15	55	8
249	66	1515	12,500	0.56	109	0	0	0	x	x	3	3	0	5	37	4
250	78	"	"	—	"	x	0	0	0	x	3	4	0	3	15	2
251	90	"	"	—	"	0	x	0	x	x	5	5	x	5	19	2
252	102	"	"	—	"	x	0	0	0	x	6	3	x	7	7	2
253	114	"	"	—	"	0	x	0	x	1	28	16	0	5	3	1
254	6	1821	1,820	660.	180	3	4	x	0	x	1	5	1	0	0	0
255	18	"	"	130.	"	0	1	0	x	3	9	6	1	x	0	1
256	30	"	"	9.8	"	0	0	0	0	7	18	2	0	0	98	12
257	42	"	"	2.9	"	0	0	0	x	2	9	2	0	x	106	18
258	54	"	"	—	"	0	0	0	x	x	7	x	0	7	134	16
259	6	1919	340	120.	300	3	6	1	x	1	1	5	2	0	0	0
260	18	"	"	24.	"	x	2	0	0	2	2	3	1	0	1	x
261	30	"	"	1.8	"	0	0	0	0	x	12	0	0	0	153	9
262	42	"	"	—	"	0	0	0	0	0	7	2	0	2	88	14
263	54	"	"	—	"	x	x	0	0	x	3	2	0	7	16	6

TABLE III—*Continued*

Haul No.	Depth	Local Sun Time	Irrad. at Surface	Irrad. at Haul Depth	Irrad. 30 min. before	Centropages typicus			Calanus finmarchicus					Metridia lucens		
						Ad. ♂	Ad. ♀	Imm.	Ad. ♂	Ad. ♀	Cop. V	Cop. IV	Cop. III	Ad. ♂	Ad. ♀	Imm.
	m.		*μw*	*μw*	*per cent*											
264	6	2022	0	0	(28 μ W at surface)	5	11	1	0	1	1	5	1	0	1	0
265	18	"	"	0		1	4	0	x	1	1	2	1	0	9	1
266	30	"	"	0		0	0	0	0	x	5	x	0	0	62	2
267	42	"	"	0		x	0	0	x	3	6	3	0	1	19	4
268	54	"	"	0		0	x	0	0	x	5	2	x	7	17	6
269	6	2126	0	0	No light	5	6	0	0	x	1	8	3	0	2	x
270	18	"	"	0	" "	1	3	x	x	1	x	2	1	0	2	x
271	30	"	"	0	" "	0	0	0	0	3	9	x	x	x	141	21
272	42	"	"	0	" "	0	0	0	1	1	3	2	0	2	28	9
273	54	"	"	0	" "	x	0	0	0	x	6	4	0	6	18	9
274	6	0337	0	0	No light	2	7	x	0	x	1	7	2	0	x	0
275	18	"	"	0	" "	x	x	0	0	1	1	3	x	0	60	3
276	30	"	"	0	" "	0	0	0	0	2	8	2	0	2	52	3
277	42	"	"	0	" "	0	0	0	0	1	7	3	0	4	21	5
278	54	"	"	0	" "	0	x	0	0	3	7	3	0	7	27	6
279	6	0428	124	33.	2	2	6	1	0	1	1	10	2	0	0	0
280	18	"	"	5.8	"	0	1	0	0	1	3	2	1	0	11	2
281	30	"	"	0.6	"	0	0	0	0	x	4	1	0	1	38	13
282	42	"	"	—	"	x	x	0	x	2	4	1	0	11	10	4
283	54	"	"	—	"	0	x	0	0	2	5	3	x	8	22	9
284	6	0542	2,360	640.	47	3	5	1	0	1	1	10	1	0	0	x
285	18	"	"	110.	"	x	1	0	0	1	2	1	x	0	x	1
286	30	"	"	12.	"	2	2	x	0	1	6	8	2	0	36	11
287	42	"	"	3.8	"	0	0	x	0	x	2	1	0	13	19	15
288	54	"	"	—	"	0	0	x	x	x	1	1	x	11	6	3
289	6	0739	8,930	2,400.	86	1	3	1	x	1	1	6	1	0	x	0
290	18	"	"	420.	"	x	x	x	0	1	1	1	1	0	0	1
291	30	"	"	46.	"	0	0	0	0	1	6	1	0	0	1	1
292	42	"	"	14.	"	0	0	0	x	x	2	2	0	0	105	32
293	54	"	"	2.4	"	0	0	0	x	0	2	2	0	3	29	7

From Fig. 3 it will be seen that at Station 1287 the adult females of *Metridia lucens* constitute the most numerous group (diagram plotted on half scale) of all the copepods. This group is distributed from 18 meters down to 114 meters, the greatest numbers occurring at the 54-meter level. The depth of maximum abundance of the adult males of this species is also 54 meters, but the range does not extend upwards so far and the animals are relatively more numerous at the lowest depths. All the immature *Metridia*[3] taken together show a maximum at 30 meters, thus exhibiting a tendency to inhabit the upper water layers more commonly than the adults. These observations on *Metridia* agree with Bigelow's conclusions (1924) that the level of plurimum abundance of this species as a whole in the Gulf of Maine is 50 to 150 meters.

Centropages typicus occurs in very much smaller numbers than either *Metridia* or *Calanus*. However, the tendency to concentrate toward the surface is very marked. This is in agreement with previous observations and is undoubtedly correlated with the warmer water found at the surface (Bigelow, 1924, and see below). The maximum numbers were taken at the 6-meter level, for both males and females, although the females were almost equally numerous at a depth of 18 meters. Immature individuals were present at the 6-meter level only.

Calanus finmarchicus is represented chiefly by immature forms, particularly copepodid stage V. The adult males occur in very small numbers at irregular intervals but increasing toward the lower limit of the range investigated (cf. Russell, 1928). The adult females show a maximum at 30 meters. The copepodid stages V and IV are found throughout the vertical range with marked maxima at 114 meters, but individuals of stage IV are relatively more numerous in the surface layers. Copepodid stage III is decidedly concentrated toward the surface. Great irregularities in the vertical distribution of *Calanus* in the Gulf of Maine have been reported previously. The present observations are consistent with Bigelow's conclusion (1924) that this species is far less plentiful in the surface stratum than below 10 meters, at this time of year.

From observations made on the penetration of light into the water at this station (Clarke, 1933), the depths at which intensities of 1000 microwatts, 100 microwatts, etc., occurred have been calculated and these are indicated by horizontal lines at the corresponding depths in

[3] The numbers of immature individuals in the cases of *Metridia* and of *Centropages* were ordinarily too small to warrant division into age and sex groups. However, this was done for Stations 1285 and 1286 in the case of *Metridia*. I am indebted to Miss Mildred Campbell for working out this further analysis. The immature *Calanus* were divided into age groups for all hauls, following the stages described by Lebour (1916).

Fig. 3 (cf. Russell, 1933).[4] The relationship between the distribution of the copepods and the intensity of irradiation may thus be readily studied. It is seen, for example, that the zone of maximum abundance for adult *Metridia* exists in a region where the intensity of irradiation is between 1 and 10 microwatts. It must be borne in mind, however, that only blue light was measured. If the irradiation over the whole spectrum had been measured, the values obtained would have been greater and the change in light intensity with depth would have been found relatively more rapid, since blue light penetrates the farthest. Moreover, we do not know how this region of the spectrum compares with other regions in stimulating efficiency for copepods. The sensitivity of crustacea in general has been found to extend over a broad range but with a maximum usually in the yellow-green (cf. Bertholf, 1932; Lumer, 1932).

Measurements were made of the lengths of 25 or more individual copepods from each of the groups which were sufficiently numerous. Russell (1928) has shown that measurements of this sort may serve to identify seasonal broods. This information would therefore be useful

TABLE IV

Cephalothorax length of Metridia lucens adult female

Length	Night Hauls			Day Hauls				
	Haul 219 6 m.	Haul 220 18 m.	Haul 223 54 m.	Haul 246 30 m.	Haul 247 42 m.	Haul 248 54 m.	Haul 250 78 m.	Haul 253 114 m.
mm.								
1.743	—	—	—	—	1	—	—	—
1.785	—	—	—	2	2	—	—	—
1.828	—	—	1	—	2	1	—	2
1.870	3	—	—	7	3	5	1	3
1.913	3	2	4	12	13	16	—	5
1.955	2	5	2	10	8	21	3	8
1.998	7	4	4	7	12	24	4	6
2.040	6	5	7	6	4	14	10	3
2.083	1	4	1	2	1	8	7	6
2.125	2	2	6	2	3	3	5	5
2.168	—	2	—	—	—	2	6	5
2.210	1	1	—	1	1	4	6	2
2.253	—	—	—	1	—	2	6	4
2.295	—	—	—	—	—	—	2	—
2.338	—	—	—	—	—	—	—	1
No. measured	25	25	25	50	50	100	50	50
Av. length	2.001	2.037	2.020	1.965	1.958	2.001	2.114	2.046

[4] For comparing light intensities 1 microwatt may be taken as approximately equal to 3 meter-candles as given by Russell, since his calculations have been based on the data of Poole and Atkins (cf. Clarke, 1933).

for comparison with subsequent investigations. Furthermore, it was desired to inquire whether size varied with depth within each group (cf. Gardiner, 1933). The frequency distributions of the lengths of the copepods are given in Tables IV, V, and VI together with the average lengths. The length of the cephalothorax (including lateral spine, if present) is the dimension considered and not the total length, since the former measurement can be made more quickly and accurately. Both the cephalothorax length and the total length (to the end of the caudal furcæ, but not including the furcal setæ) were measured for 100 individuals of adult female *Metridia lucens* and their ratio was found to be very nearly constant. The average cephalothorax length was 2.001 mm. and the average total length was 3.018 mm., giving an average ratio of 2 : 3 for this group.

From Table IV it may be seen that in the case of the adult females

TABLE V

Cephalothorax length of Metridia lucens and Centropages typicus

Length	Metridia lucens						Centropages typicus	
	Adult Male			Immature			Adult Male	Adult Female
	Haul 247 42 m.	Haul 250 78 m.	Haul 253 114 m.	Haul 247 42 m.	Haul 250 78 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 244 6 m.
mm.								
1.063	—	—	—	—	1	—	—	—
1.105	—	—	—	—	—	—	—	—
1.148	1	—	—	—	—	—	—	—
1.190	—	—	—	—	—	—	—	—
1.233	1	—	—	1	—	—	5	—
1.275	8	4	4	—	—	—	4	3
1.318	14	14	6	2	1	—	5	1
1.360	1	3	7	4	2	2	8	—
1.403	—	—	5	2	3	—	2	3
1.455	—	1	3	5	3	—	1	8
1.488	—	3	—	5	10	5	—	3
1.530	—	—	—	3	4	9	—	6
1.573	—	—	—	1	—	3	—	1
1.615	—	—	—	—	—	2	—	—
1.658	—	—	—	—	—	—	—	—
1.700	—	—	—	—	—	—	—	—
1.743	—	—	—	—	—	—	—	—
1.785	—	—	—	1	1	—	—	—
1.828	—	—	—	—	—	—	—	—
1.870	—	—	—	1	—	1	—	—
1.913	—	—	—	—	—	1	—	—
1.955	—	—	—	—	—	2	—	—
No. measured	25	25	25	25	25	25	25	25
Av. length	1.296	1.342	1.356	1.466	1.458	1.583	1.319	1.445

of *Metridia lucens* the individuals in the deeper layers tend to be longer than those nearer the surface although the differences are very small. In the upper layers the average size is slightly greater at night than during the day. If the difference be regarded as significant, it could be interpreted as due to the presence at night of large individuals which had migrated up from the deeper layers. In the case of the adult males and the immature animals of this species there is a similar increase in the number of large specimens at the deeper levels (see Table V). For *Calanus finmarchicus* the data (Table VI) reveal a more pronounced grading effect. Here the difference in the lengths of the age stages may be clearly seen. In addition it will be noted that within each group the frequency distribution definitely moves in the direction of greater length with increasing depth. This observation agrees with that made

TABLE VI

Cephalothorax length of Calanus finmarchicus

Length	Adult Female			Stage V			Stage IV			Stage III
	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.
mm.										
1.148	—	—	—	—	—	—	—	—	—	1
1.190	—	—	—	—	—	—	—	—	—	5
1.233	—	—	—	—	—	—	—	—	—	5
1.275	—	—	—	—	—	—	—	1	—	6
1.318	—	—	—	—	—	—	—	—	—	5
1.360	—	—	—	—	—	—	—	—	—	3
1.403	—	—	—	—	—	—	—	1	—	—
1.445	—	—	—	—	—	—	—	—	—	—
1.488	—	—	—	—	—	—	—	—	—	—
1.530	—	—	—	—	—	—	1	1	—	—
1.573	—	—	—	—	—	—	5	—	1	—
1.615	—	—	—	—	—	—	7	4	—	—
1.658	—	—	—	—	—	—	5	1	1	—
1.700	—	—	—	—	—	—	4	2	5	—
1.743	—	—	—	—	—	—	1	6	3	—
1.785	—	—	—	—	—	—	2	3	2	—
1.828	—	—	—	—	—	—	—	2	6	—
1.870	—	—	—	—	—	—	—	4	3	—
1.913	—	—	—	—	—	—	—	—	2	—
1.955	—	—	—	4	—	1	—	—	1	—
1.998	—	—	—	3	—	—	—	—	—	—
2.040	—	—	—	2	2	1	—	—	—	—
2.083	—	—	—	2	1	—	—	—	—	—
2.125	1	—	—	2	1	—	—	—	—	—
2.168	2	—	—	1	2	1	—	—	—	—
2.210	1	—	1	3	4	3	—	—	—	—
2.253	2	—	—	3	1	1	—	—	—	—

TABLE VI—*Continued*

Length	Adult Female			Stage V			Stage IV			Stage III
	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.	Haul 247 42 m.	Haul 253 114 m.	Haul 244 6 m.
mm.										
2.295	3	—	—	1	2	4	—	—	—	—
2.338	—	—	—	—	2	5	—	—	—	—
2.380	2	4	—	1	1	1	—	—	—	—
2.423	2	2	1	3	4	3	—	—	—	—
2.465	—	3	2	—	2	3	—	—	—	—
2.508	2	—	1	—	1	—	—	—	—	—
2.550	2	1	—	—	1	—	—	—	—	—
2.593	1	3	1	—	1	1	—	—	—	—
2.635	4	2	1	—	—	1	—	—	—	—
2.678	1	4	2	—	—	—	—	—	—	—
2.720	1	1	—	—	—	—	—	—	—	—
2.763	—	3	2	—	—	—	—	—	—	—
2.805	1	1	5	—	—	—	—	—	—	—
2.848	—	1	1	—	—	—	—	—	—	—
2.890	—	—	3	—	—	—	—	—	—	—
2.933	—	—	—	—	—	—	—	—	—	—
2.975	—	—	1	—	—	—	—	—	—	—
3.018	—	—	2	—	—	—	—	—	—	—
3.060	—	—	—	—	—	—	—	—	—	—
3.103	—	—	—	—	—	—	—	—	—	—
3.145	—	—	—	—	—	—	—	—	—	—
3.188	—	—	1	—	—	—	—	—	—	—
3.230	—	—	1	—	—	—	—	—	—	—
No. measured	25	25	25	25	25	25	25	25	24	25
Av. length	2.445	2.589	2.766	2.152	2.305	2.328	1.644	1.707	1.787	1.263

by Gardiner (1933) for stage V of this species and indicates that the tendency is of general application for the other stages.

Data on the vertical variations of the following physical and chemical factors were secured by Dr. A. C. Redfield: temperature, salinity, pH, oxygen, nitrate, nitrite, and PO_4. This information is presented graphically in Fig. 4. The temperature—which changes so abruptly between 10 and 20 meters—is probably the most important of these as an agent in determining the vertical distribution of the plankton organisms under consideration (cf. Bigelow, 1924).

It was not possible to obtain measurements of the abundance of the phytoplankton at this station, but observations were made in this vicinity about three weeks later by Gran (1933). He found that the phytoplankton maximum occurred at 20 to 30 meters. The dominating species were *Ceratium fusus*, *Pontosphæra Huxleyi*, and germinating cystæ of *Gonyaulax orientalis*.

Diurnal Migration

In Fig. 5 the changes in the vertical distribution of the adult females of *Metridia* during the 48-hour period comprising Station 1287 are represented diagrammatically. Since this is the most numerous group of copepods, more reliance can be placed upon these observations than upon those of other groups. The first series of hauls made early in the afternoon of July 10 showed that these animals were inhabiting the water from 30 meters down to 54 meters, with maximum abundance at this lower depth. As the afternoon progressed and night came on, their range was extended upwards to the 6-meter level. At the same time the zone of maximum abundance rose gradually. At 21 o'clock the greatest numbers were taken at 30 meters and at 4 o'clock the next morning the maximum occurred at 18 meters. The

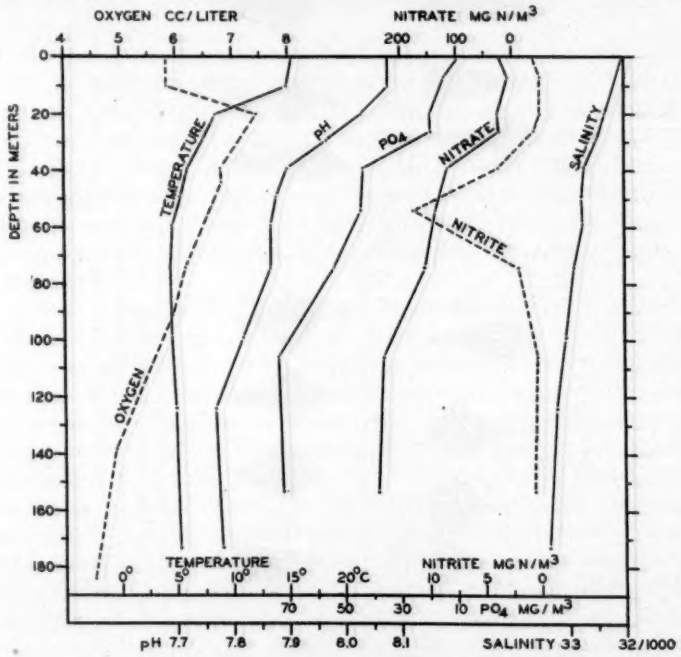

FIG. 4. Variation of physical factors with depth.
Station 1287 (except oxygen which is for Station 1285).

The pH measurements were made by a spectrophotometric method using cresol red as an indicator. The pK value of cresol red was taken as 8.13 and corrected for salinity on the assumption of a salt error of − 0.27. The solutions were measured at a temperature of 20° C. The values are not corrected for the change in temperature.

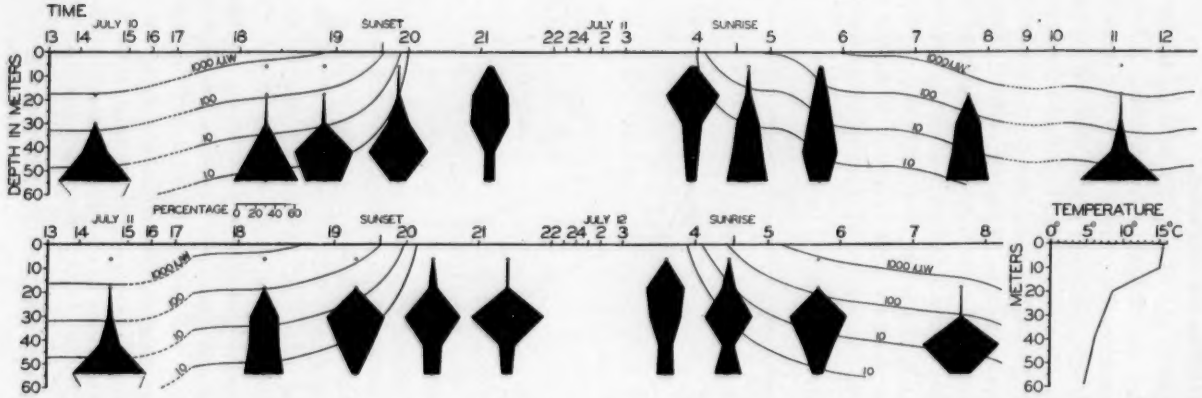

FIG. 5. The diurnal migration of the adult females of *Metridia lucens* during the 48-hour period of Station 1287 (July 10, 11, and 12, 1932). The position of the maximum early each afternoon is shown to be 54 meters, and not lower, by the deep series made immediately afterwards (see Table III). The changes in the light intensity are indicated by the lines representing the depth at which 1000 microwatts, 100 microwatts, etc., occurred. (One microwatt may be taken as approximately equal to three meter-candles as used by Poole and Atkins, 1929.) Where the lines are broken, a shortening of the time scale is indicated merely; observations were not discontinued during these periods. The temperature curve for this station is reproduced in the lower right-hand corner.

next series of hauls revealed the beginning of the downward migration. It is seen that the level of maximum abundance fell and the upper layers of water were deserted progressively toward noon. The vertical distribution of female *Metridia* at 0441, with a maximum indicated at 54 meters, does not conform to the gradual change of abundance which is indicated by the other series. The explanation for this is wanting; no corresponding irregularity occurred on the following morning. Early in the afternoon of July 11 the distribution of the animals was found to be very nearly the same as on the previous day. Toward night the animals migrated upward in similar fashion although there are certain differences in the configuration of the population diagrams. As before, the zone of maximum abundance reached the highest level (18 meters) just before dawn. The downward migration ensued as on the previous day, although here again certain differences in distribution do appear.

From the observations of the irradiation on deck and the transparency of the water (tested five times during this station), lines of equal light intensity have been drawn in Fig. 5. An idea can thus be obtained of the actual changes in irradiation to which the organisms were subjected during the course of each day. Inspection of the diagram reveals the fact that in general the zone of maximum abundance of *Metridia* occurs between light intensities of 10 microwatts and 1 microwatt (or less) and rises and falls coincidently throughout the day (cf. Russell, 1927).

At 20 o'clock on July 10 the greatest number of copepods was found below this range of light intensity and at 6 and 8 o'clock on July 12 the maximum was above it. This might be taken to indicate that the animals could not swim fast enough to retain their position within the usual limits of irradiation at those times of day when the light is changing most rapidly. For example, it can be roughly calculated from the figure that the position of the 1-microwatt line rises about 46 meters in the hour between 19 and 20 o'clock on July 10. To keep pace with this a copepod would have to swim vertically upwards at an average speed of 77 cm./min. Parker (1902) found in the laboratory that *Labidocera* swam upwards at the rate of 10 cm./min. The speeds of ascent of the Lucerne plankton observed by Worthington (1931) are even slower than this. However, Welsh (1933) has found that *Centropages typicus* swims toward a light for a distance of 10 cm. in a horizontal trough at average speeds of 71 cm. to 136 cm./min., depending upon the intensity of the light. Such an animal would theoretically be able to swim upwards at a speed equal to this rate minus the rate of sinking due to gravity. Parker observed etherized *Labidocera* to sink

at 30 cm./min. and Gardiner (1933) has shown that large specimens of *Calanus* in an anæsthetized condition sink at 50 cm./min. If the sinking rate of *Centropages* is no faster than this, it would appear that this species could ascend at a speed as great as that at which the light changes in the water. Downward swimming at such a rate could probably be easily accomplished. It should be noted that on July 11 no tendency for the zone of maximum abundance of *Metridia* to lag behind the usual range of irradiation is indicated either in the morning or in the evening.

The total range for the population of female *Metridia* never extends above the 1000-microwatt line and it often falls short of this. This value may therefore represent the greatest intensity of light which these organisms can withstand at this period of the year. The copepods do not appear to have been restricted to regions of lower light intensities in the early morning than they were later in the day (cf. Worthington, 1931), thus furnishing no evidence that the organisms became less sensitive to light as the day progressed (cf. Russell, 1931).

The observations consistently indicate that the animals did not tend to become uniformly distributed after complete darkness (cf. Russell, 1926) but continued to migrate toward the surface, reaching their highest average level just before dawn. However, the zone of maximum abundance never rose above 18 meters and very few individuals were taken at the 6-meter level. The stratum of warm water (15° C.) present at the surface, sharply delineated between 10 and 20 meters from the cold water (5° to 8° C.) beneath it (cf. Fig. 5), undoubtedly played a large part in deterring the animals from moving much above the 18-meter level (cf. Russell, 1927; Bigelow, 1924).[5] The downward migration in the morning did not begin before the irradiation in the surface layers had become measurably intense, but soon after this a rapid descent was initiated, for a considerable movement had been accomplished by sunrise.

The behavior of the other groups of copepods is less clearly defined. At Station 1053 (see Table I) *Centropages* was caught in very large numbers in the surface layers. Just before sunrise the great majority of animals in each group was found at a depth of 4.5 meters. As the day progressed, a gradual downward migration took place until noon when the zone of maximum abundance occurred at its greatest

[5] Mr. Russell suggests an alternative interpretation of these observations. He concludes from the diagrams that by dawn the distribution in the deeper layers is tending to uniformity and that if there had been no thermocline the extension of a certain proportion upward would have produced a uniform distribution. The objection to this view is that the animals present at the 30-meter level at 21 o'clock did not spread upwards and downwards in equal numbers during the night but the great majority of them moved up to the 18-meter level.

depth of 23 meters. In each case a number of animals constituting a secondary maximum remained near the surface. The downward movement of *Calanus* during this period was of much smaller proportions. Furthermore, such migration as did occur began later than in the case of *Centropages*. This may represent the difference in time before effective intensities of light reached the deeper levels where the majority of *Calanus* were living. The hydrographic data for this station are as follows:

Depth *meters*	Temp. ° *C.*	Salinity *mille.*
0	16.81	31.97
9	16.82	31.92
17	16.80	31.96
35	16.30	31.96
52	10.60	32.24
69	—	32.31
86	5.86	32.50
104	5.17	32.68

It is evident that the downward migration of *Centropages* was not limited by these factors and that the range of *Calanus* extended right across the thermocline. Very few specimens of *Metridia* were taken at this station.

During the 24-hour period comprising Stations 1285 and 1286 (see Table II) *Centropages* exhibited no vertical migration whatever, maximum numbers being taken in the uppermost net at the four times that observations were made. On the other hand, *Calanus* was taken in every haul at this station. There were extremely few adult males, and the small numbers of adult females taken were distributed irregularly, apparently undergoing no definite migration during the period. However, the groups composed of copepodid stages V and IV respectively showed a fairly regular distribution with a definite maximum in each case. The largest numbers occurred at 95 meters at noon, at 85 meters at dusk, at 65 and 75 meters toward midnight, and at 65 meters the following morning. These forms therefore inhabited slightly greater depths at noon than at other times. The data for the various groups into which the *Metridia* have been divided permit only a similarly general conclusion to be drawn. The zones of maximum abundance for adult males and for adult females existed at 96 meters at noon and at various shallower depths at other periods of the day. The distribution of the copepodid stages is seen to be very irregular.

During the 48-hour period of Station 1287 *Centropages* was found confined to the surface layers as before. In the series made on the first day the range of the adults of this species extended to 18 or 30

meters at noon while the population was chiefly limited to the 6-meter level at other times. This statement does not hold true for the second day, however. The immature group did not leave the uppermost level at any time.

In the case of the adult females of *Calanus* there appears to have been a fairly consistent movement of the zone of maximum abundance from 30 meters at noon, to 6 meters at four o'clock the next morning, and then down to 30 meters again the following noon. After this, however, the maximum occurs irregularly at a variety of depths. Copepodid stage V is represented in every series at all depths. In many cases the population is rather evenly distributed throughout the range. The maximum for each series occurs at the 30-meter level in the majority of cases without regard to time of day. Copepodid stage IV was taken in all but one haul at this station but distribution was irregular. Maxima were found at the 6-meter level for the most part. The prominent maximum found at 114 meters for both stages V and IV may constitute a population which remained at this low level throughout each day and which is distinct from the secondary maximum usually found near the surface on each afternoon. Possibly the animals comprising the latter are those which form the single maximum found regularly in the shallow series made at other periods of the day. The group composed of copepodid stage III was characteristically found in the surface strata. The copepodid stages therefore either failed entirely to migrate, or at most, they moved downwards a short distance during the middle of the day as at the previous station.

The vertical distribution of *Metridia* tends to be more consistent than in the case of *Calanus*. The adult males of *Metridia* are confined to a relatively narrow range. Apparently no diurnal migration was carried out by this group, however, for the maximum in each series except five at this station was found at 54 meters. The pronounced migration of the adult females has already been discussed in detail. The occurrence of the zone of maximum abundance for the immature of this species defies generalization; but it may be concluded that the upper limit of the vertical range of this group exists at a lower depth during the middle of the day than at night.

Discussion

The detailed study of the vertical distribution of these various sex- and age-groups of copepods impresses one with the remarkable differences in behavior exhibited. However, such lack of uniformity among different species, within the same species, and even between different broods, has frequently been reported (*e.g.* Russell, 1928, 1932;

Worthington, 1931; and Southern and Gardiner, 1932). As an example of the differences in behavior of the same species as found by different investigators, our observations on *Calanus finmarchicus* may be compared with those of Russell (1926, 1927). This worker found that the depth of maximum abundance of this species in the Plymouth area changed from about 15 meters at noon to the very surface at night. But according to the data presented above *Calanus* was ordinarily distributed irregularly throughout the upper 50 meters of water and showed only a slight tendency to migrate. Bigelow (1924) has reported considerable lack of conformity in day and night catches of *Calanus* in the Gulf of Maine. In general he finds that ". . . the reactions of *Calanus* in their local application to the gulf result in its being far less plentiful in the surface stratum than below 10 meters or so by day, and often by night, during the half of the year when the temperature is highest and the solar illumination brightest."

Although the nature of the diurnal migration of plankton organisms varies so profoundly, and although different explanations are evidently required for the details of each individual case, still the possibility exists that certain fundamental principles can be found which underlie the phenomenon in its broad outlines. Accordingly, the results of the present investigation will be briefly examined for evidence supporting any of the theories which have been advanced previously.

The absence of vertical movements in the case of some of the animals under circumstances when other forms are migrating is itself a proof that, whatever causal factors may be operating, they are not of uniform application nor effectiveness. Thus, although change of light intensity may have caused the migration undertaken by *Metridia*, it was not equally effective in producing a vertical movement of *Calanus*. Bigelow (1924) says of this species, ". . . its absence on the surface in the regions where it swarms in deeper water is not caused altogether by sunlight, for while it probably does tend to descend during the most brilliantly illuminated hours, on several occasions we have made rich catches on the surface when the sun was high in the sky."

Let us therefore confine our search for a general explanation to those organisms which do migrate, and furthermore to those which move consistently toward the surface at night and downward in the morning. Because of their large numbers and relatively consistent behavior the adult females of *Metridia* form the group from which deduction may most legitimately be drawn in the present investigation. The description of the migration of these animals already presented reveals the fact that the vertical movements definitely coincide with the periods of day and night. Hydrographic measurements made on

the preceding days by Dr. A. C. Redfield [6] showed that no significant diurnal changes in the hydrogen-ion concentration, in the oxygen content, or in the temperature were taking place in this region. It seems justifiable to conclude, therefore, as the majority of previous investigators have done, that light is the primary physical factor controlling the vertical migration of these organisms.

The behavior of *Metridia* provides evidence that, in the present case at least, other factors besides light were operating. For, as has already been seen, these animals continue to move toward the surface even after complete darkness and are found at their highest level just before dawn. This type of behavior was found by Worthington (1931) and by Southern and Gardiner (1932) for certain freshwater forms, but is in sharp contrast with observations of Russell (1927) in the Plymouth area, in which many plankton animals were found uniformly distributed in the vertical column of water at midnight. Continued migration after dark may be due to a negative geotropism, as has been suggested by Worthington and others. The possibility of such behavior being correlated with feeding habits (cf. Worthington, 1931; and Southern and Gardiner, 1932) should be examined for *Metridia*. As stated above, the phytoplankton maximum in the present case occurred at a depth of 20 to 30 meters. The upper limit of the vertical movement of *Metridia* corresponds roughly to the upper limit of this zone, but it also corresponds approximately to the beginning of the thermocline (cf. Fig. 5). Pronounced changes in temperature undoubtedly are effective in limiting migrations (cf. Russell, 1927). Furthermore, we have very little information on the actual food of particular species of copepods. More information must be obtained before the importance of feeding reactions in this connection can be decided.

In so far as light is regarded as effective in controlling diurnal migrations, it is worth while to consider in what manner it may exert its influence. The theory that plankton animals tend to keep within a range of "optimum intensity" of light (Russell, 1926, 1927), although supported by a considerable mass of evidence, has failed to fit the facts in certain cases (cf. Worthington, 1931). Question has also been raised as to the acceptability of the "optimum intensity" theory on the basis of experimental work in the laboratory (Clarke, 1930).[7] In the present investigation, however, *Metridia* were found fairly consistently within a definite range of irradiation, as has already been pointed out.

[6] Unpublished data.

[7] Due to an error, line 26, page 128, of this report became transposed. It should read: ". . . due simply to a negative phototropism and a positive geotropism produced by the rising of the sun in the morning. . . ."

According to other theories, based in large part on laboratory experiments, light is supposed to exert its effect indirectly, by modifying the reactions of the animals to such factors as gravity and temperature (cf. Dice, 1914; Esterly, 1919; Rose, 1925). Whether the control by light is regarded as indirect or as direct, an important distinction should be made between two possible ways in which it may act, viz.: by virtue primarily (1) of its *change* of intensity, or (2) of its *absolute magnitude* of intensity (cf. Clarke, 1930, 1932). The behavior of plankton organisms may be regarded as a series of similar responses to changes in the intensity of light or as different responses to constant intensities of different magnitudes.

Since responses to change of irradiation are ordinarily of short duration, it would be necessary, in order for the first possibility to hold true, for the increase or decrease of light intensity to take place continuously at a rate above the threshold. The requisite rate of change for *Daphnia magna* may be calculated from existing data (Clarke, 1930) and is found never to be less than 16 per cent per minute. In the sea the irradiation changes at a rate which varies according to the time of day but which is ordinarily greatest at sunrise and sunset (cf. Fig. 5). In order to ascertain whether the change of light intensity in the water ever proceeds at a rate above this threshold, a calculation may be made for a time when irradiation is still measurably intense but changing at a maximum speed for that intensity. Between 1900 and 1940 o'clock on July 10 at a depth of 30 meters the irradiation was reduced from 10 microwatts to 1 microwatt. This represents an average rate of change of 4 per cent per minute. If the responses of *Metridia* are to any degree commensurate with those of *Daphnia*, it would appear that the rate of change of light in the sea at no time rose above the threshold value. Nevertheless, an upward movement of the plankton took place during this time. Ewald (1910), recognizing these difficulties, gives reasons for believing that the sensitivity of the animals is increased in nature and that even a gradual change in light intensity might evoke the responses. Judgment on this question must be reserved until more information is available.

On the other hand, if the *change* of irradiation is rejected as a source of stimulation, then the problem resolves into one of the effect of different sensibly constant intensities upon the phototropism, geotropism, etc., of the organisms. In cases, such as *Daphnia*, in which the signs of the tropisms are unchanging under all intensities of light, and in which the animal is able to adapt to light over a wide range, an appeal to these reactions can not account for vertical migration, whether the fundamental feature of it be regarded as a tendency to maintain a posi-

tion within a certain zone of light intensity or as some other type of behavior. Although these experiments have not yet been proved to give a true picture of the behavior of plankton animals in nature, carefully controlled laboratory investigations of this type have already shed much light upon various aspects of the problem. Whatever theory may be advanced to explain diurnal migration, the underlying reactions involved must be demonstrated conclusively in the laboratory before the explanation can be finally accepted.

Summary

1. Observations on the vertical distribution of copepods and measurements of the submarine irradiation were made in the Gulf of Maine during 12- to 48-hour periods to procure information on the importance of light in controlling diurnal migration.

2. Five closing nets were towed simultaneously at different depths from a single vertical cable. The method devised for allowing the nets to be open only during the 10-minute towing period is described.

3. The sources of error involved in the work at sea and in the sampling of the catch in the laboratory are discussed. The methods used allow special dependence to be placed upon differences found among the hauls within each series.

4. The general vertical distribution of the three species studied was as follows: *Centropages typicus* inhabited the stratum of water above the thermocline (10 to 20 meters), *Calanus finmarchicus* was irregularly distributed, and *Metridia lucens* occurred below the thermocline. In each case the individuals present in the upper part of the range were found to have a slightly smaller average length than those occurring at deeper levels.

5. The adult females of *Metridia* exhibited the most marked diurnal migration, the level of maximum abundance rising in the afternoon and during the night and falling in the morning. These movements were found to coincide to a considerable extent with changes in submarine irradiation.

6. The changes in the vertical distribution of the other groups of copepods were slight or quite irregular. In some cases, however, there was a definite tendency for the maximum to occur at greater depths at noon than at other times.

7. This investigation confirms the idea that light is the most important factor controlling diurnal migration. In addition, the observations are shown to have a bearing on various of the theories regarding the manner in which light exerts its effect.

I am indebted to the Woods Hole Oceanographic Institution for the purchase of the closing nets and for the opportunity to make the observations from the "Atlantis" and to the Laboratory of General Physiology, Harvard University, for facilities for the preparation of the report.

I am extremely grateful to Dr. E. J. Allen, Mr. F. S. Russell, and other members of the Marine Biological Association for the use of the facilities of the Laboratory at Plymouth and for a great deal of assistance in assembling equipment when the "Atlantis" was preparing for sea, and I wish to thank Mr. Russell in addition for valuable advice in planning the methods and program of work and for most helpful criticism of the manuscript.

I am indebted to Dr. A. C. Redfield of the Woods Hole Oceanographic Institution for furnishing me with the hydrographic data and for criticizing the manuscript, and to Mr. C. O'D. Iselin, not only for assistance in designing the closing nets but also for a great deal of help in all the operations at sea. Miss Mildred Campbell of the University of Toronto very kindly analyzed the entire catch made at Stations 1285 and 1286 for me. Mr. C. L. Wheeler and Mr. D. Lillie made the measurements of the copepod lengths. In addition, I wish to thank Mrs. G. L. Clarke, Mr. D. B. Merriman, Mr. F. Bigelow, and Mr. C. L. Wheeler for much assistance in the collection and analysis of the catch and in the preparation of this report.

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THE BUFFER CAPACITY OF SEA WATER [1]

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The titration of sea water in order to measure its excess of base, or what may be called its buffer capacity for acids, is not only of interest from the standpoint of oceanography but also of practical value in the detection of acid wastes present in small quantities (Thompson and Bonnar, 1931). Several methods have been employed. Wattenberg (1930) has described a method in which an excess of acid is added to the sea water, CO_2-free air is drawn through the boiling mixture until all CO_2 is removed, and then the water is back-titrated with standard barium hydroxide solution, using a mixture of brom cresol green and methyl red as indicator. Greenberg, Moberg, and Allen (1932) use a method of direct titration with standard acid, using methyl orange as indicator. Both of these methods, and others of which they are typical, require the use of rather elaborate precautions and of more or less cumbersome apparatus.

Of recent methods, that of Thompson and Bonnar (1931) possesses certain distinct advantages. It is rapid and employs apparatus easily handled on board ship. It is especially good in that results are practically independent of the CO_2 tension of the sample.

The principle of the method is simple. A sufficient volume of standard HCl is added to a measured portion of the sample to produce an acidity near the middle of the useful range of brom phenol blue. From this the milliequivalents of hydrogen ion per liter remaining unneutralized are calculated. This value subtracted from the quantity of H ion added per liter gives what might be called the apparent buffer capacity of the water. This value, however, must be corrected for the effect of salts upon hydrogen ion activity and upon the dissociation of the indicator. The correction is made by carrying out the same procedure on a sample of carbonate-free, approximately neutralized sea water of the same salinity as the unknown sample. Subtracting the acid required by the carbonate-free water from that required by the sample gives the buffer capacity of the latter.

It seemed to us that if standard values for the amount of acid required to bring carbonate-free sea water of any given salinity to any

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given pH could be established, the method would be made much more useful, especially since the difficult part of Thompson and Bonnar's method is the preparation and preservation of neutral, carbonate-free water. Our experience has led to the conclusion that such standard values can be obtained. Sea waters from distinctly different regions, whether clear or cloudy (provided they are filtered before use) will yield the same results within the limits of observational error, when rendered truly carbonate-free.

The method of preparing the "neutralized" water has been slightly modified. Concordant and reproducible results can be obtained only when certain precautions are rigidly adhered to. Filtered sea water, sufficient to half fill a large Pyrex boiling flask, is treated with concentrated H_2SO_4, drop by drop, until a test portion gives a color with brom phenol blue approximately equivalent to that of a Clark and Lubs standard tube of pH 3.4. The water is then boiled for about six hours under a Pyrex reflux condenser, while a stream of CO_2-free air is rapidly drawn through it. The reflux condenser is then removed, and while the water is still boiling, carbonate-free NaOH of about 0.07N strength is added in small portions until a sample of the water quickly cooled to room temperature shows a pH of 6.6 with brom thymol blue. The approach to the condition of near neutrality is followed from time to time by titrating a cooled 25-ml. portion of the water with 0.01N NaOH, using brom thymol blue as indicator. As noted by Thompson and Bonnar, the occurrence of an alkaline condition in the water at any time during the approach to neutrality vitiates the preparation. CO_2 will be taken up so rapidly that the water, even though it is acidified as soon as the slight alkaline condition is discovered, will show acid requirements that are too high.

Measurements are made, after the preparation has been cooled to 20° C., upon various known dilutions with distilled water. The following series, for example, was used: 21, 18, 15, 12, 9, 6, and 3 grams per liter of halide, computed as chlorine. Approximate dilutions are prepared and the precise chlorinity later determined by titration with $AgNO_3$. Each of several 100-ml. portions of each dilution is treated with a suitable amount of 0.075N HCl added from a micro-burette permitting a precision of $\pm$ 0.001 ml. Each of the mixtures is then read at 20° C. with brom phenol blue as indicator in a pH bicolorimeter of the Hastings type. The precision of this instrument, provided its optical system is adjusted to give perfectly uniform illumination on both sides, is far beyond the expectations of one accustomed to older pH colorimetric methods. Differences corresponding to less than 0.01 of a pH unit can be recognised and measured by the experienced ob-

server. From the readings, pH values are computed, using 3.98 as the pK_I of brom phenol blue. This "constant" varies, of course, with salinity, but if the same pK_I of the indicator is used throughout, the so-called "salt-error" is automatically eliminated. The value 3.98 is convenient because it is the one established for brom phenol blue at 20° C. in Clark and Lubs' standard buffer solutions. From the pH value and the quantity of acid added are calculated the milliequivalents of H ion neutralized per liter, or the apparent buffer capacity of the carbonate-free water. The several values for each dilution are plotted

TABLE I

"Apparent" buffer capacity of carbonate-free, approximately neutralized sea water. Milliequivalents of H ion apparently neutralized per liter.

Cl after adding HCl	pH observed after adding HCl											
	4.10	4.05	4.00	3.95	3.90	3.85	3.80	3.75	3.70	3.65	3.60	3.55
gram/l.												
3	*.048*	.055	*.061*	.071	*.080*	.092	*.105*	.121	*.139*	.156	*.179*	.206
4	.058	.065	.075	.084	.095	.107	.123	.139	.158	.177	.203	.232
5	.067	.075	.087	.097	.110	.122	.140	.157	.177	.198	.226	.256
6	*.076*	.085	.098	.108	*.127*	.137	.155	.173	*.196*	.216	.247	.278
7	.083	.093	*.109*	.120	.135	.150	*.171*	.188	.209	.235	*.267*	.299
8	.090	*.101*	.116	*.129*	.146	*.163*	.182	*.204*	.224	*.253*	.285	.319
9	*.096*	.109	.125	.139	*.155*	.174	.194	.215	*.239*	.267	.302	*.339*
10	.103	.116	*.132*	.147	.167	.185	*.205*	.228	.251	.282	*.317*	.354
11	.109	.123	.140	.156	.176	.194	.215	.239	.264	.295	.332	.370
12	*.114*	*.129*	.146	*.162*	*.179*	*.202*	.225	*.250*	*.277*	*.310*	.345	*.383*
13	.120	.135	*.151*	.170	.190	.212	*.236*	.259	.288	.319	*.358*	.398
14	.124	.140	.158	.175	.197	.220	.243	.268	.298	.330	.369	.412
15	*.128*	.145	.164	.181	*.201*	.227	.250	.276	*.307*	.340	.380	*.423*
16	.133	*.150*	.168	*.187*	.210	*.232*	.259	*.283*	.316	*.351*	.390	.435
17	.137	.153	.173	.192	.215	.240	.266	.290	.324	.360	.400	.446
18	*.141*	.157	.178	.196	*.222*	.247	.273	.298	*.333*	.369	.410	*.457*
19	.145	.160	*.184*	.200	.225	.253	*.280*	.304	.340	.378	*.419*	.465
20	*.149*	.164	*.186*	.204	*.229*	.258	*.283*	.309	*.347*	.386	*.429*	*.476*
21	.152	.166	.190	.208	.234	.263	.292	.315	.354	.394	.336	.484

against the observed pH values, and when this is done for each of the several dilutions, a contour chart involving the three variables, pH, apparent buffer capacity, and chlorinity of the acidified water, is obtained. By interpolation, apparent buffer capacity for any given pH and chlorinity can be read off.

After this method had become standardized six different collections of sea water were used for extended sets of measurements. Their sources were: Woods Hole Harbor, two samples, and one each from Narragansett Bay, Atlantic Ocean near Newport, Atlantic Ocean south

of Martha's Vineyard, and Atlantic Ocean near Bermuda. From the resulting six contour charts, the mean value of the apparent buffer capacity was computed for each of 59 points distributed over the ranges of chlorinity and pH values which, when plotted, yield the contour chart shown in Fig. 1. The computed 59 points (in italics), together with others read off from Fig. 1, are given in Table I.

The precision of these values was tested by computation of the probable error of each of the mean values entered upon the contour chart. Of the 59 points 40 had a probable error of the mean not exceeding $\pm$ 0.003, 9 of them had a probable error less than $\pm$ 0.001 and the general order of the probable error was $\pm$ 0.002. In no case

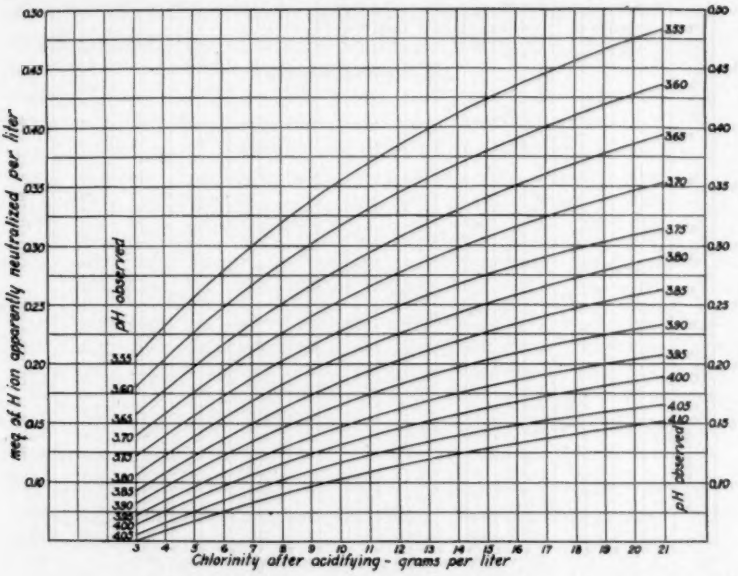

FIG. 1. The apparent buffer capacity of carbonate-free approximately neutralized sea water, used for standard correction values. Above the abscissa equal to the chlorinity of an acidified sea water sample, one selects by interpolation between the isohydric contour lines the pH observed with brom phenol blue as indicator and reads the corresponding ordinate as the correction to be subtracted from the apparent buffer capacity of the sample. These values are computed on the assumption that, for brom phenol blue,

$$pK_t = 3.98 - 0.007(t - 20^\circ \text{ C.}).$$

did the probable error exceed $\pm$ 0.006 and the less consistent results were all in a range of pH values more acid than pH 3.70, a part of the chart not recommended for use in more precise measurements, for reasons to be discussed presently.

The final test of the accuracy of these values is their actual use as corrections for measurements of the buffer capacity of sea water. If accurate they should give results on any one sample of sea water, both with and without the addition of distilled water, and at any observed end-point of titration within the chosen pH range, such that the ratio of the buffer capacity (obtained with the use of the carbonate-free correction value) to the chlorinity of the water is constant. This has been found to be the case, in repeated trials, within the limits of observational error. An example of such a test of the method is shown by the results given in Table II.

TABLE II

Titrations of the same sea water sample undiluted and diluted with distilled water

Chloride content	Titration end point	Uncorrected buffer capacity	Correction from contour chart	Corrected buffer capacity	Ratio $\frac{BC}{Cl}$
gram/l.	*pH*	*meq./l.*	*meq./l.*	*meq./l.*	
17.38	3.77	2.394	0.280	2.114	0.1217
17.38	3.79	2.368	0.264	2.104	0.1210
11.98	3.75	1.707	0.247	1.460	0.1219
9.88	3.74	1.439	0.233	1.206	0.1219
5.94	3.87	0.846	0.128	0.718	0.1210
4.05	3.835	0.608	0.115	0.493	0.1217

The selection of an appropriate end-point for the titration of sea water, when brom phenol blue is the indicator, must be a compromise. Greenberg, Moberg and Allen (1932), applying the theory of titration to the problem of methyl orange titration of the total carbonates of sea water, compute the correct end-point as pH 4.35, and use 4.5 in practice. The steepest slope of the titration curve is in the region of pH 4 to 5. This we have found to be the case by the use of the glass electrode described by Taylor and Birnie (1933). This instrument was particularly suitable for the purpose because measurements are taken in a small closed chamber which prevents loss of CO_2. Two titration curves are shown in Fig. 2. They make it obvious that the most sharply defined end-point is at approximately 4.5. Here, however, the effects of carbonic acid are considerable, and loss of CO_2 makes the colorimetric pH estimation very difficult. Indeed, carbonic acid exerts a measurable effect upon the result unless the end-point is distinctly more acid. The true dissociation constant of H_2CO_3, though unknown, is of the general order of pK_a 3.5, according to several observers (Michaelis, 1926). But if the titration is arbitrarily carried to a pH of about 3.4–3.3, that is, beyond the possible effects of car-

bonic acid, the uncertainty of the colorimetric determination is greatly increased. Readings with brom phenol blue in this range show a comparatively large observational error.

A working compromise between these various objections is reached by selecting the end-point so as to be between pH 3.70 and 4.00, a

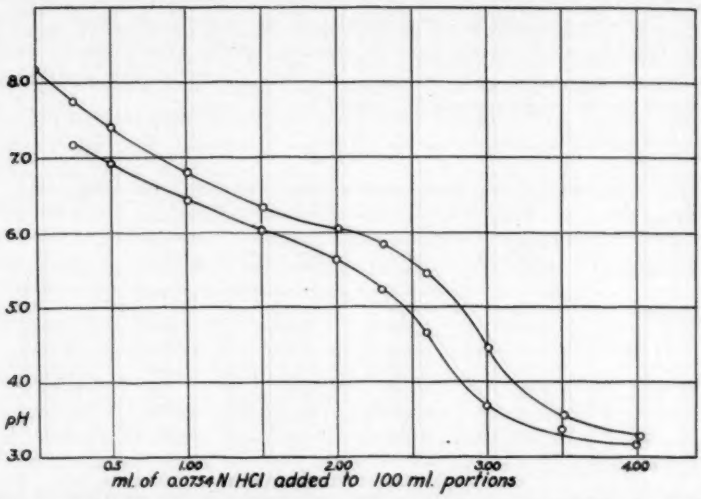

FIG. 2. Sea water titration curves. The pH measurements were taken with a glass electrode in a closed chamber. Each point was obtained with a separate 100-ml. portion of water to which was added the volume of 0.0754N HCl indicated. The pH measurements were made at 30° C.

Upper curve: water from near Newport; chlorinity, 18.10 gram/l.

Lower curve: Narragansett Bay water; chlorinity, 15.21 gram/l.

range within which the titration curve is fairly steep and the measurements can be made with a satisfactory degree of accuracy, while the effects of varying CO_2 tension in the sample are relatively small, affecting the result by an amount less than one per cent of the observed value. The latter fact was ascertained by measuring sea water samples which were equilibrated with laboratory air and then measuring the same samples after bubbling expired air through them. Using 3.98 as the pK_I of brom phenol blue in sea water, as we have proposed above, yields values which are somewhat higher than the *true* pH. In sea water of high salinity at 20° C., the pK_I is of the order of 3.78, as the work of Kolthoff (1930) on KCl solutions has indicated. Precise measurements in sea water of varied salinities and temperatures are desirable. Some preliminary work in this direction has been under-

taken in the laboratory of one of us, using the glass elecrode and the bicolorimeter. It is clear, however, that the end-point chosen for these titrations is sufficiently acid to avoid serious errors due to carbonic acid.

The following procedure is followed in taking the buffer capacity of a sample: It is collected in Pyrex glass, unless measurements can be made at once. Silicates from soda glass can measurably raise the buffer capacity. If it must await analysis for any length of time, it is preserved at a temperature not above 10° C. Fermentation acids can cause low results, in our experience. If any visible organic matter is present the sample is filtered. Protein and perhaps other organic matter raise the buffer capacity. Measurements are taken at or near 20° C. A 100-ml. portion is pipetted into a small Pyrex flask and HCl of about 0.075N strength is added in amount which, as estimated roughly from the salinity of the water, will bring it to the desired acidity as described above. Either preliminary trials or previous experience with water of the locality is necessary to determine the amount. The flask is stoppered and its contents thoroughly mixed and used for pH measurements in the bicolorimeter. The brom phenol blue can give reliable results only when of high purity, which can be ascertained by using it with Clark and Lubs' standard phthalate buffer solutions. Of five different manufacturers' products tested, only two were found to be within the limits of the accuracy of this method. For all the results reported here, brom phenol blue from Hynsonn, Westcott and Dunning was used.

The solution of the indicator must also be approximately isohydric with the solutions to be measured. This condition was sufficiently approximated by adding to the 0.04 per cent indicator solution, as commonly prepared with one molecular equivalent of NaOH, a further amount of 0.01N NaOH equal to one-third of a molecular equivalent. This solution gave the same pH readings in the sea water irrespective of the concentration of indicator. Indicator solutions containing either one or one and one-half molecular equivalents of NaOH failed to do so. An indicator solution containing more than one molecular equivalent of alkali is relatively unstable and should be prepared not more than three days before use.

It is not always feasible to secure temperature control at 20° C. during the pH observation. Although slight variations in temperature do not greatly alter the pH of the acidified sea water, they do affect the dissociation of the indicator to an extent exceeding the limits of accuracy otherwise attainable in this method. As an approximate correction for the temperature effect upon brom phenol blue, we have

used the formula, $pK_t = 3.98 - 0.007\ (t - 20°)$, where pK_t is the pK_I at temperature, t. This is taken from a few measurements with the use of the glass electrode and is subject to future correction.

The method of computation of results is shown by the following example. To 100 ml. of Woods Hole Harbor water was added 3.400 ml. of 0.07509N HCl, or 2.553 milliequivalents of H ion per liter of water. The resulting pH was 3.83, or 0.153 meq. of H ion in 1034 ml. of mixture. The apparent uncorrected buffer capacity is 2.553 − 0.153 = 2.400 meq./l. Interpolation from the contour chart of Fig. 1 or from Table I shows that at pH 3.83 and chlorinity of 17.8 grams/l (the value for this mixture), the correction is 0.260 meq./l.

TABLE III

Buffer capacity of surface water from the Cape Cod region

Date	Source of sample	Chloride content	Buffer capacity	Specific buffer capacity
		gram/l.	*meq./l.*	*BC/Cl*
12/19/30	Near Woods Hole	18.31	2.204	0.1203
"	" " "	18.34	2.220	0.1210
"	" " "	18.28	2.188	0.1198
"	" " "	18.28	2.176	0.1189
"	" " "	18.27	2.171	0.1188
"	" " "	18.17	2.165	0.1192
"	" " "	18.21	2.188	0.1200
7/27/32	" " "	18.05	2.161	0.1196
"	" " "	18.05	2.138	0.1183
8/30/32	" " "	18.48	2.181	0.1181
"	" " "	18.48	2.162	0.1170
"	" " "	18.48	2.176	0.1177
9/10/32	" " "	18.32	2.165	0.1182
9/12/32	" " "	18.24	2.164	0.1186
"	" " "	18.35	2.159	0.1176
"	" " "	18.24	2.160	0.1182
1/29/33	" " "	17.90	2.112	0.1181
9/16/32	Buzzards Bay	18.48	2.223	0.1203
"	" "	18.27	2.191	0.1200
9/22/32	Cape Cod Bay	18.11	2.183	0.1205
"	" " "	18.02	2.170	0.1203
"	" " "	18.05	2.187	0.1211
9/23/32	East of Cape Cod	18.22	2.187	0.1200
"	" " " "	18.21	2.189	0.1202
"	" " " "	18.20	2.184	0.1200
"	" " " "	18.19	2.184	0.1201
"	" " " "	18.36	2.200	0.1198
"	" " " "	18.35	2.202	0.1200
8/11/32	35 miles south of Martha's Vineyard	18.48	2.223	0.1203

The buffer capacity of the sample is, then, 2.400 − 0.260 = 2.140. The $AgNO_3$ titration of this water sample gave Cl 18.32 grams l, so that the ratio BC/Cl = 0.1168. The latter may be called the "specific buffer capacity."

Results of observations on various samples from the Cape Cod region and from parts of the Atlantic Ocean and the Caribbean Sea included in a recent cruise of the "Atlantis," are summarized in Tables

TABLE IV

Buffer capacity of sea water from the Gulf of Maine

Station	Depth	Chloride content	Buffer capacity	Specific buffer capacity
	meters	*gram/l.*	*meq./l.*	*BC/Cl*
1705	0	18.10	2.143	0.1184
	50	18.40	2.200	0.1196
	175	18.74	2.210	0.1179
1706	0	18.10	2.144	0.1185
	50	18.42	2.172	0.1179
	150	18.63	2.182	0.1171
1707	0	18.09	2.211	0.1222
	50	18.25	2.186	0.1198
	125	18.56	2.201	0.1186
1709	0	17.47	2.101	0.1203
	31	17.97	2.130	0.1185
1711	0	17.99	2.161	0.1201
	50	18.16	2.167	0.1193
	175	18.86	2.220	0.1177
1713	0	18.22	2.189	0.1201
	50	18.45	2.207	0.1196
	175	19.17	2.254	0.1176
1717	0	18.08	2.180	0.1206
	50	18.34	2.202	0.1201
	200	19.69	2.290	0.1163
1722	0	18.14	2.104	0.1160
	54	18.43	2.196	0.1192
	114	18.90	2.217	0.1173
	0	18.14	2.173	0.1198
	50	18.445	2.181	0.1182
	250	19.66	2.300	0.1170
1723	0	18.765	2.249	0.1199
	70	18.465	2.215	0.1200

III, IV, V, and VI. (The results in Table VI were obtained by Mr. Homer P. Smith, on board the "Atlantis.") Figure 3 shows the location of the principal stations in the investigation.

The specific buffer capacity, or the ratio of buffer capacity to the

TABLE V

Buffer capacity of Atlantic Ocean water

Station	Depth	Chloride content	Buffer capacity	Specific buffer capacity
	meters	*gram/l.*	*meq./l.*	*BC/Cl*
1462	0	20.90	2.420	0.1158
	0	20.90	2.452	0.1172
	100	20.90	2.450	0.1171
	2000	19.95	2.393	0.1199
	2000	19.95	2.371	0.1188
St. Georges Harbor, Bermuda	0	21.00	2.403	0.1144
1465	0	21.15	2.438	0.1152
	100	20.17	2.363	0.1171
1467	0	21.20	2.373	0.1120
	100	21.20	2.419	0.1140
	4500	20.17	2.378	0.1179
1469	0	21.20	2.402	0.1134
	100	21.15	2.419	0.1143
	600	20.83	2.386	0.1145
	800	20.45	2.380	0.1164
	1200	20.30	2.347	0.1157
	2000	20.22	2.338	0.1156
	3000	20.20	2.346	0.1161
	4000	20.20	2.346	0.1161
1473	0	21.22	2.384	0.1123
	100	21.20	2.374	0.1122
	500	21.07	2.367	0.1123
	1000	20.35	2.328	0.1144
	1600	20.20	2.317	0.1147
	2000	20.20	2.329	0.1153
	2500	20.20	2.329	0.1153
	3000	20.20	2.317	0.1147
1477	0	20.93	2.356	0.1125
	100	21.17	2.385	0.1126
	500	21.03	2.387	0.1135
	1000	20.27	2.319	0.1144
	1400	20.25	2.319	0.1145
	2000	20.25	2.295	0.1133
	3000	20.20	2.295	0.1136

TABLE VI

Buffer capacity of water from the Caribbean Sea

Station	Depth	Chloride content	Buffer capacity	Specific buffer capacity
	meters	*gram/l.*	*meq./l.*	*BC/Cl*
1482	100	20.855	2.406	0.1154
	500	20.375	2.367	0.1162
	1000	19.825	2.384	0.1203
	2000	19.845	2.384	0.1201
	3000	19.785	2.335	0.1180
1513	100	20.495	2.296	0.1120
	500	19.98	2.270	0.1136
	1000	19.745	2.245	0.1137
	2000	19.81	2.271	0.1146
	3000	19.81	2.271	0.1146
1527	0	20.65	2.355	0.1140
	100	20.755	2.389	0.1151
	500	19.77	2.310	0.1168
	1000	19.785	2.322	0.1174
	2000	19.82	2.335	0.1178
	3000	19.83	2.347	0.1184
1548	0	20.35	2.319	0.1140
	100	20.48	2.355	0.1150
	500	19.95	2.310	0.1158
	1000	19.74	2.298	0.1164
1581	0	20.425	2.355	0.1153
	100	20.435	2.368	0.1159
	500	20.07	2.309	0.1150
	1000	19.685	2.311	0.1174
	2000	19.755	2.298	0.1163
	3000	19.775	2.298	0.1162
	4000	19.76	2.258	0.1143
	5000	19.775	2.258	0.1142
	6000	19.775	2.229	0.1127
1598	0	20.38	2.391	0.1173
	100	20.36	2.381	0.1169
	500	20.00	2.346	0.1173
	1000	19.725	2.323	0.1178
	2000	19.77	2.310	0.1168
	3000	19.775	2.327	0.1177
			2.310	0.1168
1606	0	20.415	2.367	0.1159
	100	20.40	2.344	0.1149
	500	20.07	2.296	0.1144

chlorinity, is in general lower than that reported by Thompson and Bonnar for Pacific water of the San Juan Archipelago and nearby regions. That this difference is not due merely to variations in method is indicated by the results of measurements made by one of us on 48 samples from various bays in the Puget Sound region. These measurements were made by the same method as that used in this report and the same correction values for salt effects were applied. All the re-

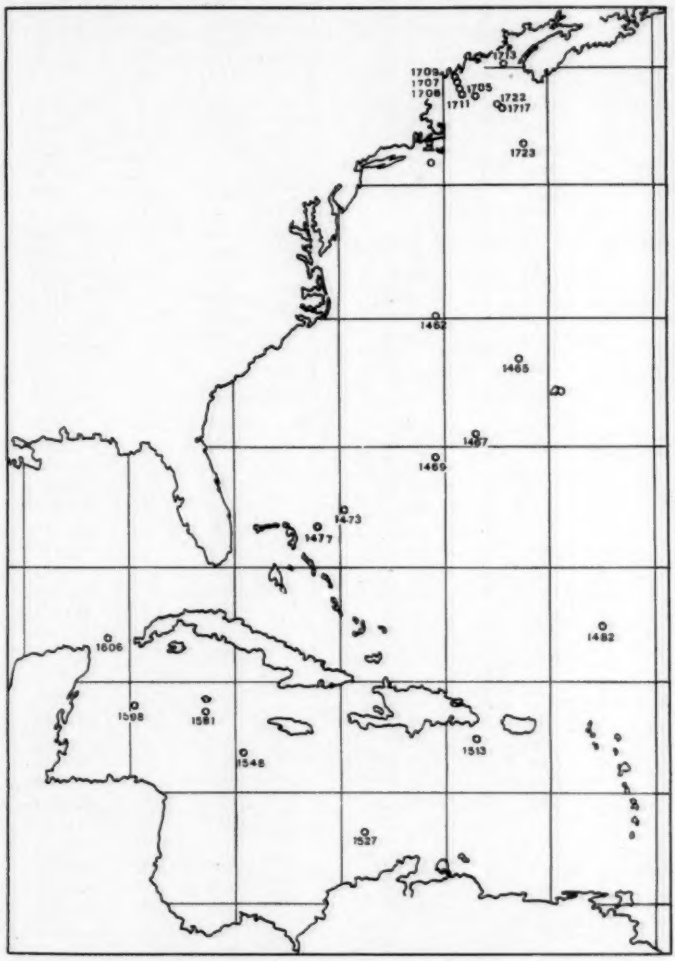

FIG. 3. Location of the principal stations.

sults fell within the range of specific buffer capacities reported by Thompson and Bonnar. It is hoped that the titratable alkalinity of Atlantic and Pacific waters can be more adequately compared in the near future. It would also be of interest to compare the ratios we have already found with those obtained in other parts of the world, but it is not yet certain in our opinion that results obtained by different methods are fairly comparable. So far as we are aware, the report of Thompson and Bonnar is the only one based upon the use of this method.

A comparison of our data with those of Wattenberg (1925–27), in the course of his extensive work in the South Atlantic on the "Meteor" expedition, shows very fair agreement. His values for the ratio of alkalinity to chloride fall mostly between 0.120 and 0.125, but using chloride per kilogram as a basis. When this is changed to chloride per liter (lowering the ratio about 2 per cent) very close agreement results with our average value of 0.119 + for water near Cape Cod.

Two general tendencies are to be seen in the above data: first, a lower specific buffer capacity in waters to the south than in those from the neighborhood of Cape Cod and the Gulf of Maine. This difference is especially noticeable in the western part of the Sargasso Sea and in the greater portion of the Caribbean. A full discussion of the significance of these differences must await further data, which we are endeavoring to obtain.

Were these variations due simply to a difference between coastal and mid-ocean water we should expect the results in the Caribbean to be similar to those in the Gulf of Maine, but it is precisely here that the difference is most marked. Wattenberg's values for "specific alkalinity" in the South Atlantic show a similar, but less pronounced, variation with respect to latitude. Averaging his results from stations in the neighborhood of 40° – 50° S. on the one hand, and those from stations 10° – 20° S. on the other, shows an increase of about 2 per cent, at corresponding depths, in the higher latitudes. A similar analysis of our more limited data reveals a difference of 4–5 per cent.

Second, there is a variation in specific buffer capacity with depth; in general, and especially at deep-sea stations, an *increase* with depth. (Wattenberg found the specific alkalinity to behave in the same manner.) In two widely separated areas, however, this variation is reversed. Both in the Gulf of Maine and in the western part of the Caribbean the buffer capacity consistently *decreases* with increasing depth. We are not yet in a position to discuss the full significance of these facts, beyond suggesting that mass movements of the water are concerned, as well as possible variations in the character of the land drainage.

It may be pointed out that the lowering of the specific buffer capacity resulting from the mixing of mid-ocean and coastal water has been apparent in several of our observations. For example, during a prolonged and violent easterly storm in April samples taken at Woods Hole had a higher chlorinity and correspondingly a lower ratio than any we had ever observed there, between 0.1138 and 0.1149.

In addition to the work here reported, many measurements have been made on samples from the Narragansett Bay region. Here, however, the buffer capacities are variable and of a different order from those observed in ocean water. The effects of land drainage, rainfall, tidal and other currents, etc., involved in a nearly land-locked body of water such as Narragansett Bay, are sufficiently complex to deserve special treatment, which will be undertaken in a later report.

Summary

1. The method of Thompson and Bonnar for measuring the buffer capacity of sea water has been modified by the introduction of the use of standard correction values to make allowance for the effects of salts upon hydrogen ion activity and upon the dissociation of brom phenol blue. These values are reported in tabulated and graphic form.

2. Various sources of error in the method are discussed and corresponding refinements of technique suggested.

3. The specific buffer capacity of Atlantic waters according to these measurements is lower than that of Pacific waters, so far as yet investigated.

4. Lower specific buffer capacities were found in the Atlantic Ocean to the southward and in the Caribbean Sea than in the neighborhood of Cape Cod and the Gulf of Maine.

5. In general, an increase in buffer capacity with respect to depth was observed, but in the Gulf of Maine and the western part of the Caribbean this relation was distinctly reversed.

6. The effects of mass movements of ocean water upon the buffer capacity appear to be traceable.

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INHIBITION OF THE STIMULATING EFFECT OF RED LIGHT ON TESTIS ACTIVITY IN STURNUS VULGARIS (STARLING) BY A RESTRICTED DIET [1]

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Introduction

In experiments on *Sturnus vulgaris* between January and March, 1930, and between December and February, 1930–31, in Hartford, Connecticut, to test the effectiveness of red, green, and white lights of equal luminous intensities in stimulating testis activity, a varied diet rich in proteins, fats, vitamins, and mineral salts, as well as carbohydrates, was used. The complete effectiveness of red light at either 2.6 or 1.7 foot candles, respectively, added for 6 hours after nightfall following a period of daylight normal for the time of year, was indicated, with such diets, in inducing spermatogenic activity outside the normal season for this bird (Bissonnette, 1932*a*; Bissonnette and Wadlund, 1931).

A further experiment, such as the one to be described here, seemed necessary to test the relations of diet to this reaction. The results of such an experiment in general indicate that such light does not prove equally effective when the diet is restricted to mashed grains, relatively poor in proteins, fats, vitamins, and salts, and quite different from the diets normal for this bird; though similar to those of grain eaters like sparrows and perhaps pigeons at some seasons.

The first set of experiments in January–March, 1930, are completely described elsewhere and will not be repeated here. They furnish further corroborative evidence of the effectiveness of the light exposures in this respect on full diets.

Studies with Adequate Varied Diets

Between December, 1930, and February, 1931, birds of both sexes were fed a diet composed of meat, bread, vegetables (green and other-

[1] Studies on the Sexual Cycle in Birds. X. Aided by grants from the Committee for Research in Problems of Sex and the Grants-in-aid Committee of the National Research Council of the United States, 1930–32, and by laboratory and library facilities of the School of Agriculture and Zoölogy Laboratories, Cambridge, England, and the Marine Biological Laboratory, Woods Hole, Mass., for which the author wishes to express thanks.

wise, cooked and raw), bacon, butter, and fruits, from the college dining-hall, ground in a meat-chopper and mixed with bran and wheat and corn meals, to make the mass mealy and not too moist to be picked up by the birds (Bissonnette, 1932*a*, and other papers; Bissonnette and Wadlund, 1931, 1932, 1933). The birds were exposed to light from electric bulbs, filtered through red and green filters, as described, and to unfiltered white light from similar bulbs. The experiments were repeated from January 24 to February 18 with the same bulbs, slightly weakened by use, and therefore of slightly less intensities. They corroborate the findings from the first experiments, though at a somewhat slower rate of activation. In each set of experiments the luminous intensities of the different colored lights were equal as they reached the roosts of the birds, at about 1.7 foot candles at first. Each set of experiments was controlled by birds similarly caged and fed but receiving no light after nightfall.

Studies on Restricted "Middlings Mash" Diet

During December and January, 1931–32, at Cambridge, England, another experiment was carried out with red and green lights of about 2.66 foot candles intensity and food rations consisting of "middlings mash" only, without the added meats, fats, vegetables, and fruits. The cages were somewhat smaller than those used most recently in Hartford but not smaller than those first used with results consistent with those from the larger cages. The more uniform light control consequent upon the smaller cages probably affected the results of the exposures to light only to make them more uniform (Bissonnette, 1931*a*, *b*).

Three birds, brought into the laboratory on December 18, 1931, and used as controls without added electric light, in an attempt to learn the avenue of reception of the light stimulus by the birds, were subjected to red light of 2.66 foot candles intensity for 6 hours per night from January 1–22, 1932. This consisted of the light from a 200-watt incandescent bulb filtered through an "H. R. Pyrometer Red" filter, from the Corning Glass Company, at 20 inches distance from the roost. The bulb emitted 2660 lumens and the filter transmitted 3.5 per cent of the luminous intensity. As the birds spent most of their time on the roost and at the food tray, set at the same level, this was the most accurate and constant means of controlling the light flux reaching the birds. Slight variations of intensity, due to different positions on the roost and with relation to it and to the light source, could not be eliminated without interfering with the freedom of action of the birds and must be taken into account in considering the results. They undoubtedly led to variations of effect on individual birds.

Two birds in a similar cage received the same intensity of green light filtered through a "Sextant Green" filter from the same company, transmitting 8.75 per cent of the luminous intensity from a 100-watt bulb at the proper distance from the roost. These birds received their green light exposures from December 18 to January 22—35 days.

No birds in either cage died between January 1 and 22, the period of experimental red light treatment. They had evidently acclimated to the confinement and to the other conditions of the experiment by that time.

The three birds from the red-light cage (2 males and 1 female) and the two from the green-light cage (both males) were killed by pressure in the hand as described in previous papers. Their gonads and other reproductive organs were taken, killed, and fixed at once in Bouin's fluid for 24 hours, dehydrated, and cut from paraffin, stained in Heidenhain's iron hematoxylin, and mounted in balsam. Sections were cut 7.5 and 10 microns thick. From these preparations photomicrographs were made at 335 diameters in all cases, for study and final comparison as a supplement to study of the slides. These are reduced in reproduction for the figures in Plate I to about 240 diameters.

After removal of the intestines, feathers, wings, and legs, the bodies of the birds with the other endocrine glands were preserved for further study of those organs.

Figures 1–9 are photomicrographs taken at 335 diameters and reduced in reproduction to about 224 ×.

FIG. 1. Testis section from a mature bird subjected to experimental red light treatment with 1.7 foot candles for 6 hours per night from December 10 till January 2, 1931 (23 days), on a complete mixed diet.

FIG. 2. Testis section from a young bird subject to similar treatment to that of the bird used for Fig. 1.

FIG. 3. Testis section from "control" bird (mature) on similar diet but without experimental red light treatment for the same time as the birds used for Figs. 1 and 2.

FIG. 4. Testis section from a mature bird subjected to red light exposures at 1.7 foot candles for 6 hours per night from January 24 to February 18, 1931 (25 days), on complete mixed diet.

FIG. 5. Testis section from mature bird subject to experimental red light exposures similar to those of the bird supplying section for Fig. 4.

FIG. 6. Testis section from a young bird used as control for birds supplying sections for Figs. 4 and 5, but without red light exposures at night.

FIG. 7. Testis section from a mature bird subjected to red light exposures at 2.66 foot candles for 6 hours per night from January 1 to 22, 1932, on a diet restricted to "middlings mash" only (22 days).

FIG. 8. Testis section from a mature bird subjected to experimental light exposures similar to those of the bird supplying the section for Fig. 7.

FIG. 9. Testis section from a bird subjected to exposures to green light of 2.66 foot candles for 6 hours per night from December 18 to January 22, 1932, on "middlings mash" restricted diet; for comparison with Figs. 7 and 8, from birds subjected to red light of equal intensity from January 1 to 22, 1932, on the same diet.

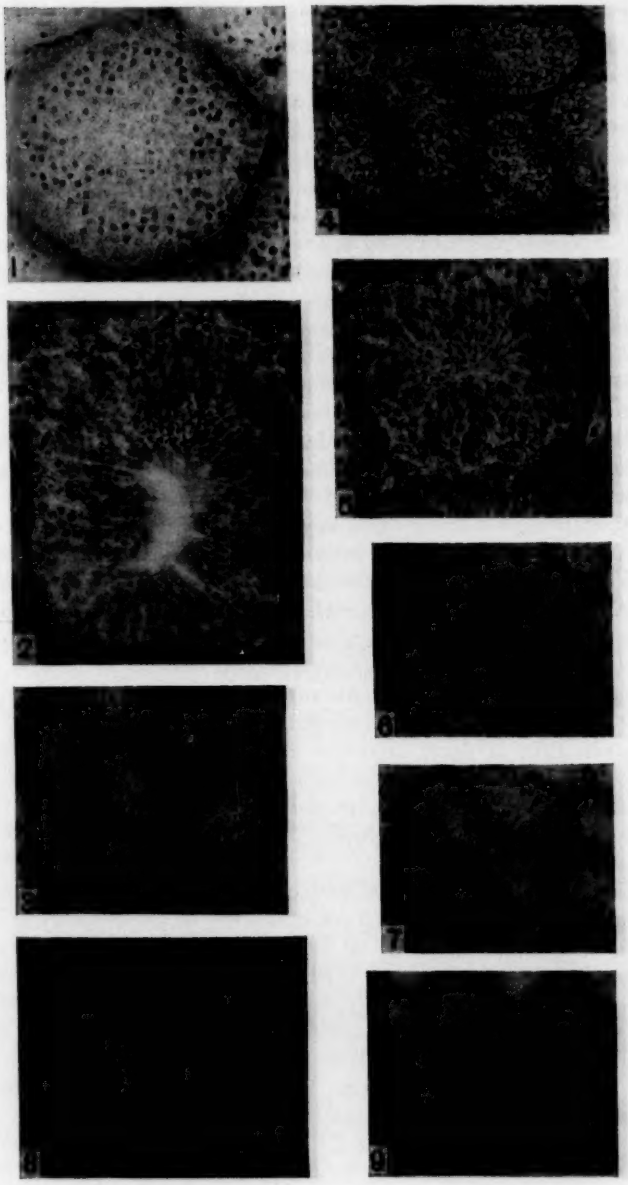

PLATE I

Results of Light Exposures on Adequate Varied Diets

After 23 days of 6-hour daily exposures to red light of about 1.7 foot candles (December 10–January 2, 1931), the testis tubules of four birds range from medium size, with early metamorphosing sperms in very small numbers (Fig. 1), to almost maximum size, with much more numerous germ-cells in all advanced stages of maturation, and numerous, almost completely metamorphosed, sperms (Fig. 2).

Control birds, under similar conditions of confinement and food, but without exposures to red light after dark, had testis tubules but slightly above the minimal normal size and without germ-cell stages more advanced than spermatogonia (Fig. 3). Conditions in the tubules of these controls were much more uniform than in the birds exposed to the red light with their variations in numbers of germ-cells of all types. Differences in amount and distribution of pigment and of interstitial cells, as between stimulated birds and controls, were similar to those previously described (Bissonnette, 1930*a*, *b*, 1931*a*, *b*, 1932*a*; Bissonnette and Chapnick, 1930, etc.) and will not be described again here.

After 25 days of similar treatment later in the season (January 24–February 18, 1931), and with intensity slightly smaller from increased use of the same bulbs in the same positions, the conditions in the testes of birds exposed to red light varied from that with the initial appearance of synizesis stages and primary spermatocytes (Fig. 4) to that with medium-sized tubules and metamorphosing sperms (Fig. 5). Controls for the same period, in some cases, had even smaller tubules and fewer germ-cells than those described above (Fig. 6).

Green light of equal luminous intensity and period of exposure induced some reduction of testis size in most cases, with reduced tubule size and fewer spermatogonia, as described in previous papers. They are not reconsidered here (Bissonnette, 1932*a*; Bissonnette and Wadlund, 1931, Figs. 28, 36).

Red light of either of these intensities induced complete spermatogenesis in these birds in 23–41 days, depending on the intensity and the relations of the birds to it, in December, January, and February. Testes of birds so treated passed the climax of activity, in spite of increasing periods of light per day, within a time which varied with the time required to induce complete activity. White light of the same intensity was also effective, but at a slower rate. Other experiments with various light combinations, intensities, and daily periods, on similar adequate diets, led to sperm formation in as soon as 18 days after first exposure to the added light, with regression following the climax within 48 days from the first exposures (Bissonnette and Wad-

lund, 1931, 1932, 1933; Bissonnette, 1932*a*). Green light of like intensities reduced testis size and activity.

These experiments showed conclusively that, on a complete mixed diet, rich in proteins, fats, vitamins, and salts, as well as carbohydrates, sexual activity in these birds is conditioned by daily period, intensity, and wave-length of the light to which they are exposed, and that increase of daily "doses" of visible long-waved light, particularly in the red wave-lengths, induces complete spermatogenic activity even in winter temperatures at the season when testes are normally at their minimum activity or even quiescent. Of the wave-lengths used, and at equal luminous intensity, red light was most stimulating, green was inhibitory, white was less stimulating than red, probably by reason of its smaller content of red wave-lengths (Bissonnette, 1932*a*; Bissonnette and Wadlund, 1931). That this was not due to differences in "heat" intensity reaching the birds, but rather to wave-length specificity, was shown by the fact that white light of equal luminous intensity to the red and green, was not intermediate, as were its effects; but was two-fifths the "heat" intensity of the green and one-tenth that of the red; but it was stimulating while green was not. The "heat" intensity was measured with a thermocouple with a variable resistance (Bissonnette and Wadlund, 1931).

Results of Light Exposures with "Middlings Mash" Diet (Restricted as to Quality but not Quantity)

The testes of the male birds exposed to red light of about 2.66 foot candles for 6 hours per night for 22 days (January 1–22) underwent but little increase in activity and germ-cell maturity beyond that of the controls described above (Figs. 7, 8). Tubules were but slightly larger. In one, germ-cells were less numerous (Fig. 7) than in controls, in the other slightly more numerous (Fig. 8). Necrotic nuclei appeared in both and no germ-cells beyond spermatogonia. Interstitial cells and pigment were like those in controls.

In a female from this cage, the oviduct was enlarged enough to be seen easily with the naked eye, and its walls considerably thickened and modified as compared with controls on normal mixed diet. In the ovary there appeared to be considerable increase in medium-sized follicles but much less change than occurs with similar light exposure on complete diets.

Testes, and the tubules therein, from the two birds exposed to green light of similar intensity on the restricted diet for 35 days (December 18–January 22, 1932) (Fig. 9), were smaller than those of normal birds

at the same time of year or than controls (shown in previous papers). Germ-cells were fewer and only a few spermatogonia were present. Pigment and interstitial cells were like those in controls or in winter birds, rather than those of January (Bissonnette and Chapnick, 1930). The necrotic nuclei, found in previous studies during the first 12 days of exposures to green light, had been cleared up by resorption before the thirty-fifth day in this experiment.

Literature and Discussion

The inhibition of the full action of red light upon testis activity, above described, with diet restricted largely to carbohydrates but with increased intensity of light, may be due to one or more of several deficiencies of diet. The experiments here described merely show that such restricted diet is incompatible with the full, or any great, effect on testis activity by increased daily periods of light exposure or of added amounts of long-waved red light. They did not differentiate or distinguish between the possible effects of the several different factors or dietary deficiencies. As shown above, the effect was not entirely eliminated by the dietary deficiencies. There was some acceleration of testis activity as compared with that in controls on an adequate diet but without added light stimulus; but it was relatively slight as compared with effects of even weaker light on the adequate diets. Further studies are planned to analyze this problem. This preliminary report is made lest the impression prevail that our studies indicate that variations of light exposure constitute the only factors conditioning sexual cycles in birds. Results of this work confirm in general the findings of many other studies on birds and mammals, that dietary deficiencies lead to interference with sexual function and rhythm, and with the action of gonadotropic hormones or other stimulators of reproductive activity.

Baker and Ranson (1932, and unpublished data) found that diet for voles may be a major modifying or limiting factor in relation to sexual cycles, either in conjunction with light cycles or without. Similar possible relation of diet to breeding cycles in birds is suggested by Bates (1908), Moreau (1931), and others cited by them, in connection with breeding records of birds in Central Africa, close to the equator, where seasonal changes of day length and of intensity of light are at, or near, a minimum. Many species have two breeding seasons each year related to the dry seasons and so to food; but the situation with individual pairs is not well known. In some species, the sex-glands of males, at least, apparently remain at breeding size and histological condition throughout the year, while nesting and laying are seasonal,

possibly in correlation with availability of insects and protein foods as well as with clear skies and changes in quality or quantity of light.

With starlings this sort of relation may obtain, so that lack of certain dietary factors may retard breeding and nesting activity till a high protein diet and certain vitamins are available for feeding the young (such as insects and other live food). But, that changes in food constituents are responsible for sexual cycles in starlings, rather than light cycles, is very unlikely in the light of the studies of Bissonnette and his collaborators, and others, in which food was identical in quality and completeness for both controls and experimental animals subjected to light changes; yet only those exposed to increased lighting of the long-waved sort underwent increased sex-gland activity (Bissonnette, 1930*a*, *b*, 1931*a*, *b*, 1932*a*, *b*, *c*, *d*, 1933; Bissonnette and Chapnick, 1930; Bissonnette and Wadlund, 1931, 1932, 1933; and others cited by them).

Ortiz (1931) reported that experimental beri-beri in cocks did not lead to alteration of sexual conditions or behavior, so lack of anti-neuritic vitamin may not have played a part in our results.

Byerly, Titus, and Ellis (1933) found that type of protein, at levels between 11.2 and 23.6 per cent in the diet, was a factor in conditioning reproduction and hatchability of eggs in fowl, and that intensity of egg-production, egg weight, and hatchability increased with a rise of percentage of protein in the food, within those limits.

Of the many studies on the relations of various vitamins to reproduction, chiefly on mammals, some are suggestive as indicating possible deficiency factors operating in our starling experiment, and may be considered here.

Sure (1924*a*, *b*, 1926*a*, *b*, 1933), among his studies of dietary requirements for reproduction in rats, found vitamin E necessary for fertility and lactation, when proteins, mineral salts, and vitamins A, B, and D were adequate. This vitamin E he found in wheat embryo among other foods, and it may have been present in the "middlings mash" used in this starling experiment, but hardly in quantity sufficient to permit fully normal sexual reaction. Sure also found vitamin B necessary for both growth and reproduction, and that skimmed milk powder, at 50 per cent level in the ration, yielded enough amino acids of excellent quality for reproduction and with 0.2 per cent of added ferric citrate gave adequate minerals. However, Sure's types of sterility were due largely to resorption of fœtuses rather than to failure of œstrus. His work suggested two fat-soluble factors in wheat oil; one, thermostable and anti-sterile, the other, thermolabile, lactation-promoting. Increase in amount of fats had no beneficial effects on lactation.

Marrian and Parkes (1929) found that female rats rendered anœstrous by vitamin B-deficiency, or by calorific deficiency in their diet, when vitamins were adequate, were immediately stimulated to œstrus and ovulation by the administration of anterior pituitary preparations. So these dietary deficiencies did not prevent gonadotropic action of anterior pituitary hormones in excess of normal. Hill and Parkes (1930) also induced œstrus, copulation, ovulation, corpus luteum formation, with pseudo-pregnancy, in female ferrets during the long winter anœstrous period, by similar preparations. But they report no spermatogenic increase and no enlargement of winter testes in treated males, which, however, copulated with treated females with the above results. Bissonnette (1932*c*, 1933) obtained similar results with ferrets, so far as copulation, œstrus, and pseudo-pregnancies in females are concerned, and some increase in testis size due partly to increase in spermatogenic activity and enlargement of seminiferous tubules and largely to increase of interstitial tissue; but no sperm production, as the result of increased lighting after dark between October and January. The results of Parkes et al., described above, indicate that excess of anterior pituitary hormone can overcome the anœstrum of vitamin B-deficiency and of inanition as well as the seasonal one of winter short days, in some mammals at least. Whether the dose required was greater than for animals not so rendered anœstrous was not determined. The similarity of their results to those of Bissonnette is suggestive of similar cause. In mammals, males are less susceptible to light induction of gonad activity than females, while in birds they appear to be quite as much affected and probably somewhat more so, as has been pointed out elsewhere (Bissonnette, 1932*c*, 1933).

Agnoli (1930) reported that vitamin E-deficiency induced changes in the seminal elements in testes of mice and that these changes could not be prevented by the administration of the lipid hormone from the anterior pituitary. On the ovary no change was induced, but the administration of the hormone led to enlargement of the uterus. Sex-glands of offspring of these vitamin E-deficient mice were modified also. These results are quite in harmony with those in the starling experiment if, as is suspected, the increase of light exposures induces increased anterior pituitary hormone in the blood and so induces sex-gland activation which is inhibited or reduced by restricted diet.

Kudrjaschov (1930) found testis degeneration in vitamin E-deficient rats of various ages with interference with normal spermatogenic function, during periods up to 300 days, leading to loss of spermatogenic cells with loss of size of accessory sex organs, while interstitial tissue was unchanged. He concluded from this that the testis hor-

mone controlling secondary sex-organs originates in the spermatogenic cells rather than in the interstitial cells. The inhibition of germ-cell maturation and multiplication in his rats is, like that induced by the deficient diet in starlings, normally stimulated by the light exposures used. In starlings, however, interstitial cells of the glandular sort appear to be lacking, and the interstitial cells that are present increase in relative amount as the germ-cell elements decrease in volume and activity (Bissonnette, 1930*a*, *b*, 1931*a*, *b*; Bissonnette and Chapnick, 1930).

Vezár (1931) reported that intra-peritoneal injections of vitamin E for over three days into infantile rats induced hypertrophy of the uterus, while subcutaneous injections of like amounts gave positive results in only 20 per cent of the cases, and oral administration led to hypertrophy in three cases. This reaction did not occur in oöphorectomized animals. Vezár and Kokas (1931) found that deficiency of vitamin E for two and one-half months caused the fur in rats to become silky, a condition relieved by administration of anterior pituitary preparations. They therefore conclude that vitamin E has an effect similar to that of anterior pituitary hormones. It seems possible, however, that it may intensify the action of the hormone already being produced while its deficiency may lessen or inhibit it, as probably occurred with the starlings on the restricted diet.

Aberle (1933) found that, in certain states of vitamin A-deficiency in rats, the standardized human placental hormone was prevented from inducing the usual pregnancy cells in the vaginal mucosa—another case of inhibition of the action of a gonadotropic hormone by dietary deficiency.

Mason (1933), in a most interesting study, reported that lack of vitamin C or of vitamin D induced degenerative changes in the testes of guinea pigs and rats which were not prevented by administration of vitamin E; that vitamins A and E are necessary for germ-cell integrity and activity, though their specific effects are different, and different from those of general inanition; that vitamin E-deficiency affects the later stages of germ-cells first, while vitamin A-deficiency permits more activity of germ-cells but depletes their numbers. Repair of rather severe inanition injury can be produced in about three to four weeks of feeding even with diets relatively free of protein. Repair of vitamin A-deficiency injury takes five to thirteen weeks. Mason has distinguished the injurious results of these different dietary deficiencies and finds that vitamin A-deficiency does not act through depletion of anterior pituitary hormone. He suggests that vitamin E is necessary to maintain normal nuclear physiological condition or some

phase of chromatin anabolism. If the same situation applies to starlings, deficiencies of one or all of these vitamins would account for failure of the stimulating light to induce much activity in the testes as contrasted with its great effect on complete diets.

Considerable work has been done on the effects of deficiencies of proteins, fats, and minerals upon reproductive capacity. Burr and Sutermeister (1932) found that on given deficient and adequate diets there were no consistent differences in reproduction and ovulation between rats kept in the dark and those given 10 minutes per day of radiation with a carbon arc sunshine lamp. Living in total darkness did not seem to affect reproduction in rats on a simple and highly purified diet. Sorour (1923) found that rats kept in darkness on a rickets-inducing diet developed parathyroid deficiency and a thyroid condition similar to that in Basedow's disease, while those kept in light had normal thyroids. This suggests a control of the thyroid by light through the hypophysis.

Clayton (1930) found that kind or source of protein in the diet was a factor in conditioning reproduction and lactation in rats at a 15 to 20 per cent level, with fats adjusted at 20 per cent level with lard (found detrimental to vitamin E and later omitted), and cod liver oil either given daily or omitted, to avoid its deleterious effect when mixed with the food. Dried egg and kidney were superior to liver and milk proteins and these to muscle protein, probably because of differences of mineral content. As vitamin E-content decreases in the order: egg, milk, round, liver, kidney, the difference could not be due to vitamin E-content alone but to differences in protein. Clayton believes the superiority of egg over other forms of protein is due to its vitamin E-content at the 20 per cent level, more adequately supplemented by minerals and other vitamins. In the raw state, kidney was most adequate for reproduction. Raw egg and raw beef round were poorly assimilated. Vegetable foods proved low in protein and in the fat-soluble vitamin B needed.

Swanson and Nelson (1933), using a 15 to 30 per cent protein diet, found dried pork muscle inadequate for reproduction in albino rats, except in two cases at the 30 per cent level, whereas beef muscle in the same proportions had no bad effect. This indicated that source and type of protein in the diet affect reproductive faculty.

Russell (1932) found that addition of fresh beef or meat scrap to a milk-wheat diet improved reproduction as well as growth rate and vigor of young, in white rats, while dried yeast instead only slightly improved reproduction. He concluded that this benefit of meats could not be chiefly due to increased calcium and phosphorus or to increase of the Ca : P ratio.

Slonaker (1931) found that efficiency of reproduction in rats was at a maximum when proteins were at the 14 per cent level in the diet and fell off in the following order of percentages of proteins: 10, 18, 22, and 26. Sterility in males was greater than in females in each group and sex ratio was below normal in all groups, with a suggested prenatal mortality greater in males than in females, postnatal mortality greater in females. Length of reproductive span, sex ratio of litters, annual number of litters and of young were in the same order related to percentages as above. In size of litter, the 10 per cent group was first, the 14 per cent next, and the others in the same order. In the first three groups males had a longer reproductive span than females; the reverse in the other groups. The period of gestation was, from shortest to longest, in the following order: 10, 14, 26, 22, 18 per cent.

Evans and Bishop (1922*a*, *b*) found length of œstrous cycles closely correlated with state of nutrition of experimental animals; that the 5-day or less œstrous cycle of normal rats on a standard diet was either lengthened or otherwise modified by starvation or by deficiency in proteins, fats, mineral salts, or vitamins; that deficiency of vitamin B or of A gave prolonged diœstrus and cornified stages (2 and 3). Hoffman (1923) confirmed this for rats on an exclusive bread diet, and Wolfe (1930) for mice on a diet deficient in mineral salts. Macy, Outhouse, Long, and Graham (1927) confirmed this for deficiencies of vitamins A and B; but Parkes and Drummond (1926), Coward (1929), and Coward, Morgan, and Dyer (1930) did not.

Lin (1931) and Wu and Chen (1929) found that vegetarian rats grow slower and fail to reach as great a maximum weight as those on omnivorous milk-wheat diet and also suffer from a mild form of nutritional deficiency. Lin, Tsai, and Wan (1932) found that œstrous cycles were not affected by mild nutritional deficiency, but were lengthened by very poor diet with prolonged diœstrous intervals, but no change in cornified stages.

The foregoing studies indicate that both quantity and quality of proteins must be adequate to permit proper reproduction in rats, mice, and guinea pigs, and, probably, in other animals. Quantity of proteins was not determined in our deficient diet nor in the complete ones; but it was doubtless deficient both in quantity and quality in the former and adequate in the latter.

Bryan and Gaiser (1932) reported that both diet and increased amounts of anterior pituitary hormone are factors in increasing growth rate in adolescent male rats, and that both at the optimum together give maximum growth response. Diet affected the response to anterior pituitary hormone.

Agduhr (1931*a*, *b*) found that, with dogs, cats, and white mice, addition of medicinal cod liver oil prevented œstrus and ovulation and, in some cases, affected the testes. Taken in connection with the destructive effect of red light upon vitamin D in irradiated ergosterol, discussed elsewhere, this suggests the possibility that cod liver oil with vitamin D may be antagonistic to red light in action on sex glands and sexual cycles and that in some way it is tied to the calcium balance so that vitamin D raises the calcium in the system and interferes with the anterior hypophysis, the ovaries, and, less often, the testes.

Taken together, the preceding studies indicate a profound influence of diet upon reproductive rhythm and capacity through the presence or absence of vitamins A, B, D, E, through general inanition from nutritional deficiency, and through different proportions of proper proteins, fats, and mineral salts. Such deficiencies appear to induce conditions in testes similar to those shown by starlings and ferrets in winter, with the germ-cells limited to spermatogonia. These end results are induced in different ways by the different types of deficiency. Inadequate diets prevent or limit the action of anterior pituitary hormones much as they prevented or reduced the effect of red light in these experiments with starlings.

It is of interest here that Küstner (1932) found that anterior pituitary gonadotropic hormones were apparently destroyed by ultra-violet light, while their effectiveness was intensified by radiation with red light, so that the Aschheim-Zondek test for the presence of the gonadotropic hormones in urine could be read off in immature mice after 60–72 hours if the mice were kept in red light during the test, instead of the usual 96 hours under ordinary lighting conditions. Tests indicated that the hormone was present in both active and inactive forms in prolan, and that red light converted the inactive into the active form, increasing the active amount, and that ultra-violet rays reversed this action without destroying the hormone.

Whether the normal effect of added red light on starlings is to convert inactive gonadotropic hormone into its active form, thereby increasing its effect upon the gonads and indirectly on the accessory sex-organs, or to stimulate the hypophysis to increased secretion or liberation of the hormone into the blood stream to be effective, still remains an open question requiring further analysis. But that this effect is lessened by a restriction of the diet is beyond serious question.

Summary and Conclusions

1. The effects on testis activity of red light at about 1.7 foot candles for 6 hours per night with starlings on a complete and varied diet are

compared with those of red light at about 2.66 foot candles for the same time per night on similar birds fed a diet restricted to "middlings mash," poor in proteins, fats, vitamins, and mineral salts, and with controls on the complete diet without added red light.

2. The experiments were carried out in January and February for 22, 23, and 25-day periods.

3. On the complete diet, red light at 1.7 foot candles for 23 days led to large increase in testis size and in tubule cross-section, with metamorphosing sperms, in January; and, in February, with the same bulbs at slightly weakened intensity due to use, to a slightly less increase and to less advanced germ-cell stages, in 25 days.

4. On the restricted diet, the increase in testis size, tubule section, and spermatogenic activity was relatively slight, leading at most to multiplication of spermatogonia.

5. This is taken to indicate that restricted diet is a factor limiting the effectiveness of the light stimulus, or response to it, so far as sexual activity in male starlings during the long winter period of quiescence is concerned.

6. Cases from the literature are cited, in which lack of vitamins, proteins, fats, and mineral salts, either singly or in combination, has interfered with sexual activity or with the action of growth and gonadotropic hormones of the anterior pituitary, and their bearing on these experiments discussed.

7. Cases are cited and discussed in which long-waved light has increased the amount and effectiveness of anterior pituitary hormones and short-waved rays have decreased them.

8. It is concluded that increase of exposures to long-waved light can only be completely effective in inducing sex-gland activity, in starlings and at least some other animals, when the diet is rich in vitamins, proteins, and fats, as well as carbohydrates. The relative effects of deficiencies in each of these different requirements were not investigated.

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CONDITIONS AFFECTING THE RESPONSE OF THE AVICULARIA OF BUGULA

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INTRODUCTION

The avicularia, or "bird's heads," on the *Bugula* which abound in the waters around Woods Hole present an interesting example of primitive muscular mechanism—interesting both from the point of view of physiology and from that of their utility to the colonies in which they are found. Two species of *Bugula* (*B. flabellata* and *turrita*) are common in this vicinity. Each zoöid consists of a shell into which the polypid with its ring of ciliated tentacles can on appropriate stimuli be retracted. Normally each zoöid has also an avicularium growing on the outside of its shell as an appendage apparently quite distinct from the rest of the organism. Indeed active avicularia are sometimes found on dead zoöids in which the rest of the organism is already decomposed. The avicularium has an extraordinary resemblance to the head of a hawk or eagle, the hooked beak and the articulation of the lower jaw or mandible being almost identical in shape with those of the bird (Figs. 1 and 2). Usually the beak is held wide open, the angle between the jaws being about 150°. To quote Harmer (Cambridge Natural History, Vol II, p. 484), ". . . A great part of the head is filled with a strong muscle, whose fibers exhibit a distinct transverse striation, and converge into a median tendon. . . . The muscle serves to close the jaws. . . . The lower jaw is opened by means of a pair of muscles. . . ."

"Within the jaws, in the region which we may term the palate, is a rounded knob, which bears a tuft of delicate sensory hairs, which doubtless enable the avicularium to recognize the presence of any foreign body. The closure of the mouth may, indeed, be instantaneously induced by touching it with the point of a needle."

When a branch from a colony is placed in sea water under a microscope the avicularia are seen to sway back and forth on their stalks in the sagittal plane, usually remaining from two to ten seconds in the backward position and five to twenty seconds in the forward position; occasionally the entire cycle is as brief as five seconds or less. At intervals of a minute or two the beak closes with a quick snap, and

after remaining closed for from one to five seconds or so, opens again, whereupon the avicularium continues swaying as before. Usually the closure occurs during the forward swing of the swaying motion. As far as was observed, the swaying always ceases while the beak is closed.

As to the biological significance of this structure, Hertwig (p. 323)

FIG. 1. Microphotograph of avicularium when beak had been closed and swaying stopped by injection of fresh water. The branch of the colony was broken close to the attachment of the avicularium; this afforded an exceptionally clear background.

FIG. 2. Diagram of avicularium with beak open, showing tuft of hairs on rounded knob. The position of the muscle in the crown of the head is shown by radiating lines.

states that it has been seen to seize and hold small animals, and suggests that its function is to feed the colony. In Parker and Haswell (p. 352) a defensive function is suggested. Harmer (*loc. cit.* pp. 485–487) says, "In the course of some observations . . . on *Bugula calathus* at

Naples, a fine hair offered to a small colony was seized with such force by the avicularia that the entire colony was lifted out of the water by the hair. The same colony had captured (1) a small *Nereis*, which it held with several of its avicularia; (2) an Anisopod Crustacean, 2½ mm. long; and (3) a small Amphipod, which was held by one of its antennæ. . . . Curiously enough, however, an avicularium did not necessarily close even when part of a captured animal was actually in its mouth. The avicularia made no attempt to place themselves in an advantageous position for catching fresh parts of the *Nereis*, which they might easily have done. The avicularia which had captured prey remained motionless. The others moved backwards and forwards . . . ten times in 3/4 to 1 minute, snapping their jaws perhaps once in that time. The two Crustacea were still retained by the avicularia two days later. On the next day they had both disappeared; but the colony had again caught the *Nereis*, which had previously effected its escape with the loss of nearly all its tentacular cirri . . . it cannot be doubted that the avicularia can prevent inquisitive worms from straying at will over the surface of the colony."

In 1905, in the laboratory of the U. S. Fish Commission at Woods Hole, an attempt was begun in collaboration with Dr. E. B. Krumbhaar, to ascertain the nature of the stimuli and environmental conditions which give rise to the closure of the beak. The speed of closure is characteristic of the contraction of striated muscle. The question arises as to how the muscle is caused to contract. Bearing in mind the theory that the avicularia aid in feeding the colony, we selected a nutrient material for our first test. A branch from a colony was placed in sea water in a shallow glass dish under a microscope and the avicularia were watched for some time. One selected for observation was seen to snap shut at intervals of approximately 100 seconds. A mussel was then broken open and some of the fluid from its shell drawn into a fine glass pipette. This material was then squirted into the water surrounding the avicularium under observation. Immediately the interval between successive closures fell to about 8 seconds, but soon increased again to approximately its previous value.

As a control, sea water was similarly squirted at the structures under observation, in order to ascertain whether the effect had been due to the current of fluid. No increase in frequency of closure appeared to follow this procedure.

In another test, a solution of cane sugar was injected into the field. No response to this procedure was observed.

Further experiments have been conducted at the Marine Biological Laboratory at intervals since the preliminary observation, in the quest

for more light on the problem. The material has been *Bugula turrita*, usually scraped from the piles of the wharf, but in some experiments small colonies grown in the laboratory on glass slides by Dr. Young were used. The latter had the advantage of freedom from foreign material with which the colonies are usually encumbered.

Method

The method which proved most satisfactory was to break a small branch from the colony, large enough to include a few dozen zoöids, and place it on the floor of a Stender dish with a small glass weight resting on the stem or base of the branch to steady it and keep the zoöids under observation fairly still in the microscope field while the test substance was being introduced. Under a low power microscope one or more of the avicularia were observed and their activity when undisturbed was carefully noted. Attention was chiefly focussed on the frequency of closure, but in some of the experiments the character of the swaying was also noted.

After the effect of the test substance had been observed it was freely washed out with sea water and the material was examined until it was evident that typically normal conditions were re-established before another test was made.

The time of closure and opening was usually recorded by rapid reference to the second hand of a watch. In a few experiments it was recorded on a smoked drum with a signal magnet operated by hand. This procedure enabled the observor to watch the material without interruption.

Results

As already stated, in the first experiment mussel juice strikingly increased the frequency of closure. In many subsequent experiments the same procedure produced the same result with perfect regularity. A typical example follows. A field was found in which 10 avicularia could be seen at once. Of these, 2 held their beaks closed; the remaining 8 were swaying with their beaks open. Occasionally one was seen to snap shut. Later when 7 were in the field, 3 or 4 of them were observed to snap during a 50-second interval while they were continuously watched. After this, mussel juice, drawn directly from the shell, was injected. Apparently all avicularia in sight snapped within 1 or 2 seconds of the time the injection entered the field. A single avicularium was then watched and the times of successive closures recorded as follows: 8, 8, 10, 15, 20 seconds. Two or three minutes later the frequency of closure was still above normal. Twelve minutes after the injection the frequency had returned approximately to normal.

Another effect of these injections was a marked increase in the swaying of the avicularia. Similar results were frequently obtained with the juice of the clam and quahog. In two experiments fresh cow's milk was injected. In one of these an apparently positive result of increased frequency of snapping was observed; in the other, no effect. Again dilute solutions of sugar in sea water appeared to be wholly without effect.

In one experiment a solution of 20 per cent ethyl alcohol and sea water was injected. The injection was immediately followed by vigorous withdrawal of the tentacles of the polypids into their shells. No closure of the avicularia was observed, and about a minute after the injection all motion ceased. The avicularia had stopped swaying, and all the beaks remained wide open.

The injection of a solution of sodium chloride of much higher concentration than sea water was followed by closure of the beaks and cessation of swaying. Instead of opening as usual, the beaks remained closed. After 5 minutes the salt solution was washed away with fresh sea water, and 2 minutes later the avicularia were again swaying in normal fashion and occasionally snapping shut as usual. In a number of subsequent experiments this effect was tested by merely concentrating the sea water by boiling down to about 40 per cent of its original volume. Considerable volumes of this concentrated sea water were injected into the field of observation. Since the injections were diluted by the sea water in which the preparation was immersed, it was impossible to make these observations quantitative. It was evident, however, that with strong concentrations the avicularia regularly closed their beaks, stopped swaying and remained motionless with their beaks closed for an indefinite time. With weaker concentrations a fairly rapid series of closures would be induced, similar to those which followed injections of mussel juice. Sometimes after two or three closures the beak would remain closed as in the case of stronger injections. In other cases, after remaining closed for half a minute or so, the beak would gradually open and resume its normal activity. This effect is presumably explained as the result of return to normal concentration of sea water through diffusion.

In three experiments the effect of dilution was tried by injecting fresh water into the field. The effect was somewhat similar to that of concentration, in that a small quantity of fresh water seemed to cause frequent closures of the beak, whereas larger quantities caused the beaks to close and remain closed and motionless (Fig. 1). As in the case of concentrated salt solution, even after the beaks had remained closed and motionless for several minutes, they could be made to open and resume their normal swaying by returning them to sea water.

Chemical Stimuli

It was suggested by Professor G. H. Parker that the effects of various ions should be investigated. The following substances were tried: —acid (HCl), alkali (NaOH), $MgSO_4$, $CaCl_2$ and KCl. None of these

TABLE I

Reagent	Concentration of injected fluid	Effect on avicularia	Effect on polypid tentacles
NaOH	0.002 N	Slightly increased swaying, no closure.	Not noted.
	0.1 N	Delayed permanent closure.	Activity, then retraction.
HCl	0.001 N	No effect.	Increased activity.
	0.002 N 0.01 N	Rapid closure, then opening with cessation of swaying.	Not noted.
	0.02 N	Closure, followed by slow incomplete opening.	Not noted.
	0.1 N	Rapid permanent closure.	Retraction.
$MgSO_4$		Slight, doubtful increase in frequency of closure.	Not noted.
$CaCl_2$	Concentrated.	Doubtful increase in closures.	Not noted.
	Dilute.	Slight, doubtful increase in closures.	Not noted.
KCl	1 gram in 20 cc. sea water.	No closure, cessation of swaying.	Not noted.
	Diluted till not refractile in sea water.	Rapid closure, then opening with cessation of swaying.	Retraction.

produced such striking effects on the avicularia as did the juice of the mussel and clam. In some experiments the activities of the tentacles of the polypids, as well as those of the avicularia, were noted. The results are shown in Table I.

It should be noted that the concentration given is that of the injected fluid, which necessarily became diluted to an unknown extent on injection into the field. In the case of $CaCl_2$, its solution in sea water

resulted in the formation of crystals. To avoid mechanical effects, these were allowed to settle and the clear fluid above was used. This appeared highly refractile when dropped into sea water, and in some experiments, in order to minimize osmotic effects, it was diluted with fresh water till the refractile effect disappeared.

The most striking result in this series was the effect common to alcohol, KCl, and weak HCl. Whereas normally the avicularia only cease swaying when the beaks are closed, these three reagents caused cessation of swaying with the beaks open.

In one experiment some of the mussel juice was tested with cresol red and found to have a pH of about 7.4. The pH of sea water, measured with thymol blue, was found to be about 8.2 to 8.4. From this it might be questioned whether part of the reaction of the avicularia to mussel juice could be due to its acidity. The differences in the reactions to the two agents, described above, renders this highly improbable.

Several experiments were tried with solutions of egg albumen. The albumen was stirred up in about twice its volume of sea water. After stirring it did not appear to go completely into solution, but fluid drawn from the upper layer, *i.e.* above the albumen lying visibly in the bottom of the dish, when dropped into sea water showed distinct refractile effect, and greater density than sea water, by sinking to the bottom. Injection of this solution caused a marked positive response. Several avicularia started closing fairly regularly at intervals of from 3 to 15 seconds and continued to do so for some time. This procedure was repeated several times in different experiments and always produced similar results. In one instance the increased frequency of closure was observed for as long as 4 minutes after the injection. This persistence of the increased frequency of closure seemed to place the response in the class with the response to injections of mussel juice, rather than with the response to concentrated sea water, whose effect, when marked, terminated with permanent closure and cessation of activity. In one experiment the solution of egg albumen and sea water was diluted with fresh water till it appeared less dense and slightly less refractile than sea water. This diluted solution appeared to cause a slight doubtful response in a few avicularia somewhat similar to that of the potassium chloride. Some of the albumen solution was then carefully mixed with fresh water till it seemed to have about the same density as sea water. An injection of this solution was followed by occasional closures of one or two avicularia, but again it was doubtful if this was a true positive response. Certainly it was not nearly so marked as that produced by a dense solution of albumen.

To eliminate the possible effect of solids, some of the mussel juice was filtered. The filtrate caused a visible refractile effect when dropped in sea water. This filtrate, when injected into the field caused in the avicularia a positive response almost as pronounced as that which occurred when mussel juice with all its solids was injected. *Limulus* blood injected into the field caused no apparent effect. In another test, filtered *Limulus* blood was injected. Within 40 seconds 3 avicularia were seen to snap, which suggested a positive response, but must be considered doubtful. Apparently the most pronounced positive response is caused by solutions having a marked density, such as the juice of the mussel and the clam or fairly concentrated solutions of albumen.

That this effect may be due in part to a high osmotic pressure of the solution is suggested by the fact that closure is induced by concentrated salt solution. On the other hand, since the response to strong salt solution usually differs from that evoked by albumen, in that the result is usually a permanent closure or at least a closure more prolonged than normal, rather than an acceleration of the frequency of normal closures, and since albumen solution is characterized more by density than by a high osmotic pressure, it may be inferred that the mass of the molecules may be a significant factor rather than the osmotic pressure.

Thermal Stimuli

The effect of heat was tested by injecting sea water warmed to between 28° and 36° C. This caused acceleration of swaying, and possibly a slight increase in closure.

Electrical Stimuli

Electrical stimuli were tried in several experiments. Electrodes were applied to opposite sides of the field of observation. In some experiments galvanic currents from a 3-volt battery were employed; in others faradic stimuli from the secondary coil of an inductorium, rapidly interrupted (tetanizing) currents being delivered by means of a vibrating armature. Neither galvanic nor faradic currents of any strength employed appeared capable of stimulating the closing muscle of the avicularium. The beginning of the constant current or of the series of induction shocks was always promptly followed by retraction of all the polypids into their shells, but the avicularia seemed to continue swaying without giving any visible response. On one occasion when the current was strong enough to liberate bubbles of gas in the sea water one avicularium was seen to close when one of the electrodes was brought very close to it. On another occasion faradic stimulation,

much stronger than was required to induce retraction of the polypids, appeared to cause a slight delayed response in the avicularia. Since in no instance did the closure of the beak follow immediately on application of the electrical stimulus, but if at all, only after a strong current had been flowing for several seconds, it may be inferred that such stimulation as occurred was due to the electro-chemical effects of the current passing through the sea water and not to a direct electrical excitation of the muscle. It is remarkable that this striated muscle, which is so readily responsive to certain types of stimuli, should be so refractory to electrical stimuli whose effect on the muscles of the adjacent polypids was so marked.

Mechanical Stimuli

It was only after most of the experiments thus far described had been performed that I became aware of the existence of the tuft of hairs within the angle of the jaws, described by Harmer. These structures are small and can only be seen upon careful examination with fairly high power. The opinion expressed by Harmer, that these hairs are sensory receptors for tactile stimuli, suggested a new line of inquiry. The most effective substances thus far tried had been the juice of the mussel and the clam, which contained a considerable amount of solid material whose contact with the hairs might have caused the response.

Two procedures were adopted to test the question whether the hairs might serve as receptors for tactile stimuli or indeed might prove to be the only effective means of evoking a response in muscle. One was the comparison of the effects of the entire mussel juice, including its solids, with that of the filtrate, from which the solids had been removed. The other was to introduce insoluble solids which could fairly be assumed to be chemically inert. The result of the first procedure has already been described. Filtered mussel juice proved effective in increasing the frequency of closure of the avicularia, although probably not quite so effective as the whole mussel juice including the solids. From this and from the effect of albumen solution it may be inferred that mechanical contact of solids with the hairs is not the only possible source of stimulation.

In several experiments the direct effect of inert solids was tried. The substances employed were suspensions of clay which had been washed repeatedly in sea water to remove any soluble substances which might confuse the effect, and MgO, the crystals of which are apparently quite insoluble in sea water. A considerable number of injections of clay were made, and a majority of these were followed quite promptly by closure of one or more avicularia under observation. A few tests were negative, but a majority seemed to show a fairly definite positive response. A certain percentage of negative results

in such a test is to be expected, since it is a matter of chance whether any particles of clay come in contact with the hairs in a particular avicularium.

Several tests were made with suspensions of MgO crystals. In some ways this material was more satisfactory than clay, since the crystals formed a more even suspension with less tendency to obscure the field. The fairly rapid rate with which they sank facilitated observations in the following manner. A drop of the suspension was let fall from a pipette directly over the field of observation. The crystals would then sink in a few seconds to the bottom, and the moment at which they reached the focal plane of the microscope, including the avicularia under observation, could be observed and the effect noted. Several times this procedure produced a fairly striking positive result, of which the following is typical. When a cloud of crystals reached the focal plane, 4 out of 5 or 6 avicularia in the field snapped almost simultaneously and 3 of them repeated snapping at intervals of 10 seconds or less thereafter. When the MgO had been freely washed away, the avicularia returned to their usual slow frequency of closure. In some experiments no response to the injection of MgO crystals was seen, although in at least one instance the crystals appeared to come in contact with the hairs as the avicularia swayed forward. The failure in these cases may well be explained on the supposition that the mass of the crystals which collided with the hairs in these instances was too small to provide an adequate stimulus. In one experiment the crystals which formed when anhydrous $CaCl_2$ was dissolved in sea water appeared to produce a similar positive effect on two of the avicularia in the field.

In spite of the difficulty of insuring positive mechanical stimulation by the injection of solids, enough instances of response followed these injections to warrant the conclusion that solid masses in some way tend to cause closure of the beaks. The best evidence of this sort came through a fortunate accident. In one experiment, while an avicularium was well placed in the field for careful observation, a small planarian was seen to crawl along the branch of the colony. As the worm approached the avicularium its anterior end was seen to come squarely in contact with the tuft of hairs in the angle of the wide open jaws. Instantly the jaws snapped shut upon the head of the worm. The immediate response to this contact seemed to leave little doubt that the hairs had indeed acted as receptors to the tactile stimulus, thus confirming the assumption expressed by Harmer, as did occasional closures on contact with other foreign bodies. Clearly contraction of the closing muscle can be evoked by adequate mechanical disturbance of the tuft of hairs within the jaws.

Summary

1. Attempts have been made to throw light on the function of the avicularia of *Bugula* by noting the effect of various physical and chemical agents on their activity. Both concentration and dilution of sea water caused prolonged closure of the beaks, with cessation of swaying. Slight concentration and dilution caused rhythmic closing and opening of the beaks, the periods of closure being relatively longer than normal. Strong alkali (NaOH) and acid (HCl) caused lasting closure, although in the case of alkali the reaction was less prompt than with acid or concentrated sea water. Weak alkali accelerated the swaying, but did not cause closure. Weak acid caused rapid closure followed by opening and cessation of swaying. Inorganic salts did not cause any pronounced reaction, although KCl caused a response similar to weak HCl—a single closure followed by cessation of closure and of swaying of the avicularia, the beaks remaining open.

2. The fluid drawn from the shell of the clam or the mussel regularly caused a great increase in the activity of the avicularia, and in the frequency of closure. Albumen solution caused nearly as marked a reaction, but its effect largely disappeared when the solution was diluted to the same density as the sea water in which the *Bugula* lay. Sugar solution produced no effect. The effect of mussel juice was not wholly due to solids in suspension, since it was nearly as marked in the case of the filtrate from which the solid particles had been removed.

3. Mechanical stimuli due to injections of inert solids in suspension in sea water are effective in causing closure. These stimuli probably act on the tuft of hairs in the angle of the jaws. This was strikingly shown by the prompt closure when the head of a worm came in contact with the hairs. Harmer reports a similar response to touching them with a needle.

4. No response could be produced by electrical stimulation, even when the current caused all the polypids to be promptly retracted. A slight delayed response occasionally following a very strong stimulus was probably due to electrochemical effects.

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MARINE BACTERIA AND THEIR RÔLE IN THE CYCLE OF LIFE IN THE SEA

III. The Distribution of Bacteria in the Ocean Waters and Muds About Cape Cod[1]

HERBERT W. REUSZER[2]

Introduction

A considerable number of investigations concerning the number of bacteria and their distribution in sea water have been made. Among the earliest of these was that of Russell (1892, *a* and *b*, 1893), who conducted systematic studies of the distribution of bacteria in the Gulf of Naples. He found bacteria in small numbers to be uniformly distributed in the water at distances of 3 to 15 kilometers from shore and at depths ranging from 50 to 1,500 meters. It was found by others (Cassedebat, 1894; de Giaxa, 1899) that while the water of harbors and bays might contain large numbers of bacteria, this number, as determined on the ordinary solid bacteriological media, became very small at comparatively short distances from shore. Bassenge and Fischer (1894, *a* and *b*), who investigated the water over a wide area of the Atlantic Ocean, found bacteria in larger numbers than have any other investigators. The largest numbers, however, were found near land or in the presence of large quantities of floating sea weed. Lloyd (1930), in her investigations in the Clyde Sea area, followed the distribution of bacteria at five stations during the course of a year. In the rather shallow waters of this region, the surface water was found to be richest in bacteria, the number decreasing with depth but usually increasing slightly again at the bottom. The numbers in the surface water fluctuated considerably but no definite seasonal trend could be detected; below the surface the numbers were remarkably constant throughout the year. Numerous, usually somewhat fragmentary investigations, ranging from polar to tropical waters, have almost invariably shown small numbers of bacteria to be present in the sea water. Among these may be cited those of Frankland and Burgess (1897), Levin (1899), Otto and Neumann (1904), Gazert (1912), Berkeley (1919) and Lipman (1929). The results of recent investigations such

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[2] The author wishes to acknowledge the assistance of Mr. Charles E. Renn in certain of these experiments.

as those of Cholodny (1929) and Rasumow (1932), in the direct microscopic examination of sea water, indicate that bacteria are much more abundant than the cultural methods used by previous investigators have shown.

Studies concerning the bacteria of marine bottom deposits have been few. Russell (1892 *a*) studied the distribution of bacteria in the mud of the Bay of Naples simultaneously with his studies of bacteria in the water. This included mud at depths ranging from 50 to 1,100 meters below the surface of the water and extending to 15 kilometers from land. He found that down to depths of 200 meters, the number of bacteria decreased with distance from land, the range being from 70,000 to 285,000 bacteria per cubic centimeter of mud. Below 200 meters the number remained more constant. He had no satisfactory explanation to offer for this distribution. In work of the following year (1892 *b*) on the mud of Buzzards Bay on the Massachusetts coast, he found smaller numbers of bacteria than at Naples, a fact which he correlated with the lower water temperature in Buzzards Bay. The number of bacteria in sand bottom was much lower than in mud. Russell considered the mud to have a bacterial flora indigenous to itself, since two or three species of bacteria normally found only in the mud made up 30 to 50 per cent of all the bacteria present. Fischer (1894 *a*) and Gazert (1912) appear to be the only investigators who have studied the bacteria in deep sea deposits. Neither found bacteria to be very abundant in deposits of this nature. From many of their samples no bacteria developed. Their results are in direct contrast with those obtained from marine mud near shore. In the chalky mud of the flats about Andros Island in the Bahamas, Drew (1913) found bacteria to be present up to the number of 160,000,000 per cubic centimeter. Lipman (1929) and Bavendamm (1932) likewise found very large numbers of bacteria in these deposits. Lloyd (1931), in her systematic investigations in the Clyde Sea area, found bacteria up to 300,000 per gram of dry mud in the surface layer of mud. The number of bacteria decreased with depth and in the lower depths of the mud remained fairly constant. In the upper layers of mud there was in certain cases a bacterial zonation which was probably correlated with the stratification of the mud into organic and inorganic layers as noted by Schokalsky (1928) and Moore (1931).

The literature of marine bacteriology has recently been reviewed extensively by Benecke (1933), but the brief review above is sufficient to indicate that the work in general has been fragmentary in nature. There have been few studies of the distribution of bacteria in the sea in which an attempt was made to establish definitely the significant

factors with which the observed changes in bacterial numbers might be associated. The supplementary data obtained by various investigators is in most cases so scanty as to make it impossible to correlate the results of one investigator with those of another.

The studies reported below represent preliminary results in a more extensive study of the occurrence of bacteria in the sea and the factors influencing their distribution. The areas investigated were the continental slope south and east of Cape Cod, the Gulf of Maine, and certain of the bays adjacent to Cape Cod. The station locations, with the exception of those in Buzzards Bay and the Gulf of Maine, are given in Fig. 1. The location of the Gulf of Maine stations has been previously given (Rakestraw, 1933).

Experimental

Methods

The water-sampling bottle used in this work was similar to the evacuated glass tube used by Russell (1892 *a*), the original idea of which is ascribed by him to Massea. For breaking the tip of the inlet tube at the proper depth, a modified form of the sampler described by Wilson (1920) was used. Mud samples were obtained by means of a modified form of the sampler described by Moore and Neill (1930). The glass tube in which the sample was secured was sterilized before use and the sample for analysis taken from the center of the core. The tube was closed at both ends with a sterilized rubber stopper immediately upon reaching the surface. No sampler satisfactory from the bacteriological standpoint is available for sampling sandy deposits. The few analyses of the bacterial content of such deposits reported in this paper were obtained from samples taken from the center of the material brought up in a sampler of the clam shell or snapper type.

In the present investigation, as in most previous ones, the plate method of determining the number of bacteria was used. It should be pointed out that since not all bacteria are capable of developing on the agar plate, the results so secured must be interpreted with considerable caution, particularly in regard to the total number of bacteria found. The observations of Pütter (1924), based on the oxygen consumption of the bacteria in sea water, and the work of Cholodny (1929) and Rasumow (1932) with the direct microscopic count, all indicate that the number of bacteria actually present in sea water is much greater than the cultural methods of determining their abundance have indicated. In preliminary experiments with various media, one having the following composition gave the highest counts of bacteria and was used throughout the work:

Peptone	1 gram
Glucose	1 gram
K_2HPO_4	0.05 gram
Agar	15 grams
Sea water	1000 cc.

It is interesting to note that with the exception of the addition of glucose, this medium is similar to the one employed by Drew (1913) and which Lipman (1929) found to give higher counts than any of the other media with which it was compared. Sea water was plated both undiluted and diluted ten times, three plates of each dilution being used. The mud was plated in a similar manner, except that the moist mud was diluted 10, 100, and 1000 times. Moisture determinations were made on the mud samples and the results are reported as number of bacteria per gram of dry mud. All samples, immediately upon being taken, were placed in a refrigerator at 4–5° C. and kept at this temperature until plated. In most cases the plating took place in less than twenty-four hours after taking the sample. The plates were incubated at room temperature and counted at the end of 7 and 10 days.

Abundance of Bacteria in Sea Water

In order to determine the variability which might normally be expected in the number of bacteria in sea water, 10 samples were taken at a single station, on the 20-fathom line south of Martha's Vineyard. All the samples were taken at a depth of 10 meters, and were plated out immediately when brought to the laboratory. The number of bacteria found in each of the 10 samples was as follows: 6, 2, 3, 4, 8, 83, 10, 24, 11, 27, 18. The average number of bacteria for all the 10 samples was 19 per cubic centimeter, with a variation between the individual samples of 2 to 83 cells per cubic centimeter. It would seem, therefore, that considerable variation may be expected in the number of bacteria in samples of sea water taken from the same spot. Russell (1893) observed that an occasional sample of sea water contained much larger numbers of bacteria than was to be expected from the bacterial content of the particular locality. A single species of bacteria always predominated in such samples. While the number of colonies appearing on plates of the same series was in general quite uniform, a similar observation was made during the course of the present work. The increased number of organisms sometimes was very large and occasionally appeared on only one plate of a series. Russell explained this phenomenon by assuming that some of the bacteria in sea water exist in the form of zoögleal masses. It suggests also, however, that part of the bacteria may be associated with particulate matter in the sea and that the large numbers of bacteria in certain samples may be due to the presence of

the decomposing remains of some small marine organism such as a copepod or a chain of diatoms.

The data in Table I give an indication of the number of bacteria occurring in water receiving land and sewage drainage and illustrate the effect of local currents. Samples 1 and 2 were taken in the outer harbor of New Bedford and were very much higher in bacteria than were the three samples taken in Buzzards Bay outside the harbor. The location of these stations is not indicated on Fig. 1, but none of them were more than one or two miles from land. At the time the samples were taken the tide was rising and the direction of water move-

TABLE I

Abundance of bacteria in water and mud of New Bedford Harbor and Buzzards Bay, July 1, 1932.

Station	Location	Water depth	Number of bacteria per cc. of water or per gram of dry mud				Carbon content of surface mud	Carbon content of mud at 10 cm. depth
			Surface water	Bottom water	Top 2.5 cm. of mud	Mud at 10 cm. depth		
		meters					*per cent dry mud*	*per cent dry mud*
1	100 yds. from sewer outlet	9	1790	3230	158,500	20,100	3.37	1.21
2	Across channel from sewer outlet	8	—	4250	41,200	—	0.58	
3	Above harbor entrance	15	—	197	79,900	—	2.30	
4	Directly opposite harbor entrance	10	—	94	48,000	44,400	1.96	
5	Below harbor entrance	18	22	111	34,000	—	1.76	

ment was from Station 5 into the harbor towards Stations 1 and 2 and up the bay from Station 5 towards Station 3. The fact that the water, taken so near a source of pollution as was Sample 1, did not contain more bacteria is probably due to the fact that with an incoming tide at this time the tidal currents tended to carry the polluted water up into the harbor and left in its place the Buzzards Bay water from Station 5 with relatively low bacterial content. This is indicated by the fact that the water at Station 1, 100 yards on the bay side of the sewer outlet, contained 3,200 bacteria per cubic centimeter, while that at Station 2, approximately one mile away but in the direction of the water movement, contained 4,200 per cubic centimeter. The water in the bay itself contained much smaller numbers of bacteria. At Stations 4 and 5 there were about 100 bacteria per cubic centimeter, while

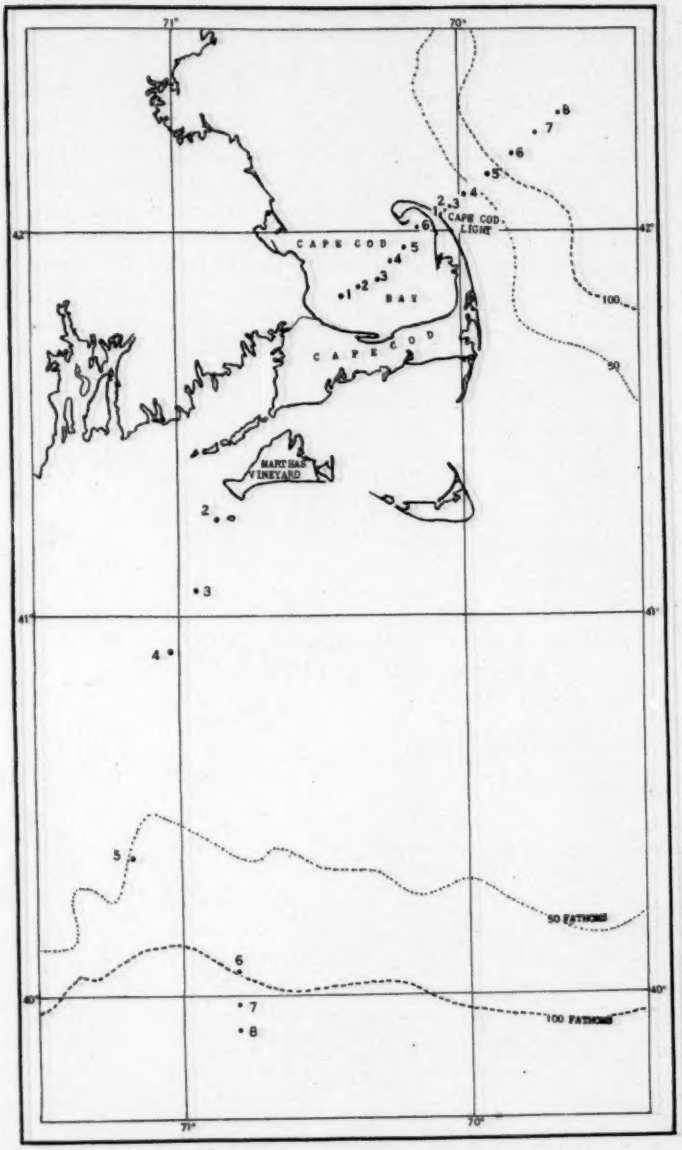

FIG. 1. Chart showing location of the stations sampled for determination of the number of bacteria in sea water and bottom deposits.

at Station 3 farther up the bay the number reached 200 per cubic centimeter. The surface water evidently contained fewer bacteria than did the bottom water. An incomplete series of surface samples was secured because of loss of vacuum by the bottles prepared for taking them.

To secure information as to the number of bacteria in water and bottom deposits of the open ocean away from the influence of land a line of stations was sampled from the southern shore of Martha's Vineyard Island to the continental slope about 80 miles to the south. The results of these determinations are given in Table II. The number of bacteria in the surface water was found to vary irregularly and did not seem to be correlated with distance from land or depth of water. While the sample taken at 8:30 in the morning contained the highest

TABLE II

Abundance of bacteria in sea water and in sea bottom deposits south of Martha's Vineyard, July 19–20, 1931.

Station No.	Depth of water	Distance from land	Hour of day when sample was taken	Number of bacteria per cc. of water or per gram of dry mud				Loss on ignition
				Surface water	Bottom water	Top 2.5 cm. of mud	Mud at 10 cm.	
	meters	*miles*						*per cent dry mud*
2	18	1.5	7:00 P.M.	65	159	—	—	—
3	38	12	5:00 P.M.	183	1900	238 (sand)	—	0.69
4	55	23	3:30 P.M.	185	—	1050 (sand)	—	0.94
5	94	55	8:30 A.M.	735	—	7700	2700	7.93
6	163	72	7:00 P.M.	27	—	1000 (sandy)	—	3.17
7	345	77	6:00 P.M.	480	—	1400	460	6.14
8	570	81	3:00 P.M.	190	—	1280	900	7.24

number of bacteria, the sample with the second highest number of bacteria was taken at 6:00 P.M. There appeared to be no definite correlation between bacterial numbers and the length of time during which the surface water had been exposed to sunshine, as was suggested by Fischer (1894 *a*). The bottom water at the two stations at which samples were obtained was much higher in bacteria than the corresponding surface water. These samples were taken very near the bottom in water of no great depth, where agitation of the surface of the bottom by water movements may have carried bacteria into the lower layers of water.

A series of observations of the bacterial content of the water and mud off the eastern coast of Cape Cod north of the Nantucket Shoals and Georges Bank was made during the summer of 1932. This line of

stations extends NE by E½E from Cape Cod Light, the sampling stations being 100 yards and 1, 2, 5, 10, 15, 20, and 25 miles from shore. As will be seen from the water depths, the ocean bottom along this line slopes gently downward to about 15 miles off shore, where it levels off at a fairly constant depth of about 245 meters. The number of bacteria found in the water of these stations at three different periods during the summer is recorded in Table III. With the exception of the samples taken near the beach, extremely few bacteria were present in the water at any time. A considerable number of the samples did not contain any bacteria capable of developing on the agar plate. The highest number found was 78 per cc. at Station 4 on August 24. The number of bacteria tended to be slightly higher at all stations at

TABLE III

Abundance of bacteria in sea water off Cape Cod Light, July 13–September 20, 1932.

Station No.	Distance from shore	Depth of water	Number of bacteria per cc. water					
			July 13		August 24		September 20	
			Surface water	Bottom water	Surface water	Bottom water	Surface water	Bottom wate.
	miles	*meters*						
1	100 yds.	5	38	2	394	793	—	—
2	1	25	0	1	—	—	—	—
3	2	36	0	0	—	—	—	—
4	5	83	1	0	78	22	—	—
5	10	159	3	0	1	1	—	—
6	15	243	0	1	12	3	0	—
7	20	246	4	0	5	—	0	1
8	25	245	—	—	5	2	0	0

this date. The number of bacteria in water one mile from shore was no greater than in water taken farther from shore. On an exposed shore such as this with the sea receiving almost no land drainage, it is doubtful if the number of bacteria in the water is directly affected by the presence of land. The larger number of bacteria in water from the beach is no doubt due to bacteria carried up from the bottom by wave action.

The bacteria in the water off Cape Cod Light may be compared with the number found in Cape Cod Bay (Table IV) and also with those for the Gulf of Maine and Georges Bank. The latter results have been discussed elsewhere (Waksman et al, 1933) in connection with other biological data secured at the same time. The water taken from the

Gulf of Maine proper, Stations 1329–1333, was, with a single exception, characterized by low numbers of bacteria similar to those found in the water off Cape Cod Light. The surface water at Station 1333 contained the exceptionally high number of 380 bacteria per cubic centimeter. No explanation for this is known except the possibility of the presence in the sample of the decaying remains of some small marine organism. The number of bacteria in the relatively shallow water over Georges Bank, Stations 1334–1336, was uniformly higher than in the Gulf of Maine. This paralleled a higher phytoplankton content in the Georges Bank water (Gran, 1933). The finding (Waksman et al, 1933) that large numbers of bacteria occur with the plankton, particularly the phytoplankton, suggests that the higher bacterial content of this water is due to increased available organic matter resulting from the high plankton content. Bacterial numbers

TABLE IV

Abundance of bacteria in sea water of Cape Cod Bay, August 24–September 21, 1932.

Station	Depth of water	Number of bacteria per cc. water			
		August 24		September 21	
		Surface water	Bottom water	Surface water	Bottom water
	meters				
1	26	8	23	—	—
2	28	19	15	—	—
3	35	17	26	—	—
4	38	9	36	2690	850
5	37	8	21	4670	720
6	26	26	23	2620	1020

did not vary significantly with depth at any of these stations. If the above explanation relating an increased bacterial content with an increased phytoplankton content is correct, then it would seem that the numbers of the two types of organisms should have a parallel variation with depth. The studies reported above were not detailed enough to determine whether or not this was the case. It is possible that with the low quantities of plankton ordinarily found in the sea during the summer months, it would be very difficult to detect variations of bacteria with depth during this time. A study of the numbers of bacteria associated with the annual spring increase of the plankton should afford results extremely interesting from the standpoint of the decomposition and transformation of the organic materials produced in lreatively large amounts at that time.

Returning to the water of Cape Cod Bay, it will be seen that the number of bacteria found there on August 24 is significantly higher than that found on the same date off Cape Cod Light but approximately equal to the number occurring over Georges Bank on August 5. At three of these stations sampled on September 21 there was an increase in the number of bacteria over that found on August 24 amounting to practically a hundred-fold in both the surface and bottom water. This increase may or may not be due to the fact that the samples of September 21 were taken between 4:00 and 6:00 A.M., while those of August 24 were taken from 7:40 to 11:25 P.M. On August 24 the bottom water at four of the six stations contained more bacteria than did the surface water. Of the three stations sampled September 21, all contained from two to six times as many bacteria at the surface as at the bottom. The only factor under observation which varied at the two sampling periods was, as noted above, the time of day at which the samples were taken. This does not seem to offer an adequate explanation for the wide variation found in the distribution of the bacteria.

Abundance of Bacteria in Sea Bottom Deposits

The mud samples of which the bacteriological analysis is reported here were obtained at the same stations as those described above for the water samples. In general no attempt was made to sample the bottom deposit at those stations where it consisted only of sand. At a few stations, however, sand samples were obtained and these are designated as such wherever they occur in the tables. The deposits examined were entirely of terrigenous nature. The material from which the bacteria living in the bottom deposits of this type secure their food comes then from two sources: (1) The organisms living in or on the sea bottom and those sinking from the water above, (2) the organic matter present in a partially decomposed state in the sediments brought in from land. The relative amount of these two sources of organic matter will vary widely depending upon local conditions. The fresher organic matter tends to accumulate at the surface of the bottom and to become incorporated into the lower layers of mud only very slowly through the action of burrowing organisms living in the marine bottom. Sand bottoms contain relatively little organic matter, but the findings of Raymond and Stetson (1931) show that over them also there occurs a small amount of organic material.

The number of bacteria found in the ocean bottom at the stations south of Martha's Vineyard in July, 1931, is given in Table II. The figures for loss on ignition of the dry mud are also given as a rough indi-

cation of the amount of organic matter in the samples. The sand samples contained fewer bacteria than did the mud samples. This is especially true of the sand taken nearest shore where there is practically no organic matter present. In the mud samples there is a progressive diminution in the number of bacteria present with distance from shore. The number of bacteria in these samples cannot be definitely correlated with the amount of organic matter present, probably because in these very finely divided sediments a considerable part of the loss on ignition is due to the loss of physically or chemically bound water. The number of bacteria diminished rapidly with depth into the mud. Later determinations on mud samples from the Gulf of Maine indicated

TABLE V

Abundance of bacteria in sea water and in bottom deposits of the Gulf of Maine and Georges Bank, August 2–5, 1932.

	Number of bacteria per cc. water							
Station No.	1329	1330	1331	1332	1333	1334	1335	1336
Depth of water in meters	256	207	230	346	230	71	74	64
Depth of sample								
Surface	10	3	4	2	380	16	92	6
50 meters	1	1	2	1	2	—	—	—
Bottom	6	0	1	1	3	25	41	27
Depth in mud	Number of bacteria per gram of dry mud or sand							
Surface 2.5 cm	5400	4700	830	3000	—	—	1280 (sand)	670 (sand)
10 cm	1720	1700	140	290	—	—	—	—

(Table V) the fact that they had a bacterial content of the same order of magnitude as the muds south of Martha's Vineyard. The figures for the number of bacteria in mud samples from Buzzards Bay and New Bedford Harbor given in Table I indicate the much higher bacterial content of sediments near land. The carbon content of these muds as determined by a modification (Tiurin, 1931) of the method of Schollenberger (1931) is given also. The determinations indicate a very close direct correlation between the number of bacteria in these samples and the content of organic matter as indicated by the carbon determination.

In the sampling method for mud, described above, the mud was obtained as a core in a glass tube. The core was removed from the tube

by pouring off the water above the mud, inserting a small piece of sterile cotton, and then pushing the mud from the tube with the aid of a wooden rod slightly smaller in diameter than the inside diameter of the glass tube. By this procedure the surface layer of mud became somewhat compressed and mixed and the extreme surface was absorbed to some extent by the cotton. The surface sample of mud represented, therefore, a composite of approximately the top inch of mud. The organic matter added to the mud from the water above tends to accumulate at the very surface of the mud in a zone probably so thin as to be measured in millimeters rather than centimeters. A change in the procedure for removing the mud from the tube was then adopted in order to study the number of bacteria in the extreme surface layer of mud. In pouring off the water above the tube about 4 cc. were allowed to remain behind. Previous platings of this water had shown that unless mixed with mud very few bacteria were present. The

TABLE VI

Distribution of bacteria and organic carbon in the mud of the Atlantic Ocean off Cape Cod Light, August–September, 1932.

Station No.	Depth of mud	Bacteria per gram of dry mud							Per cent organic carbon in dry mud		
		August 24				September 20			September 20		
		Surface	2.5 cm.	10 cm.	20 cm.	Surface	2.5 cm.	10 cm.	Surface	2.5 cm.	10 cm.
6		5,000,000	630	340	—	125,500	4,300	270	5.20	1.30	1.37
7		919,000	1695	176	—	48,800	4,900	2,390	16.66	1.50	1.77
8		23,100	1490	220	0	25,300	4,700	2,290	5.77	1.72	1.77

tube was then shaken until the surface layers of mud were in suspension. One cubic centimeter portion of the suspension was then pipetted off for bacteriological analysis, another for moisture and total carbon determination, and another for a sea salt determination. The amount of dry material in one cubic centimeter of such a suspension was usually about 60 mgm., although it varied from 35 to 90 mgm. with different samples. Of the dry material, usually one-half or more, depending upon the concentration of mud in the suspension, was sea salt. The carbon and bacteriological determinations were corrected for the presence of this salt.

The number of bacteria found in mud samples from Cape Cod Bay and the Atlantic Ocean off Cape Cod Light by the above method are given in Tables VI and VII. The carbon content of the mud is in-

cluded in these tables. The carbon content of the surface layer was considerably higher than that of any of the lower layers. In two cases values as high as 16.66 and 20.00 per cent carbon in the surface mud were found, but these values may possibly be excessive. An inspection of this data shows at once the importance of the extreme surface layer of the mud from the standpoint of bacteriological activity. The number of bacteria in the surface exceeded by far the number present at a depth of only 2.5 cm. at all stations. Not only was the surface layer rich in total number of bacteria but it also contained a much more varied bacterial flora than did the lower layers. Agar plates of samples from the 2.5 cm. depth revealed only small, grayish white, either round or lens-shaped colonies. Plates from the surface layer, on the other hand, contained many types of colonies, including

TABLE VII

Distribution of bacteria and organic carbon in the mud of Cape Cod Bay, August–September, 1932.

Station No.	Depth of mud	Bacteria per gram of dry mud						Per cent organic carbon in dry mud		
		August 24			September 21			September 21		
		Surface	2.5 cm.	10 cm.	Surface	2.5 cm.	10 cm.	Surface	2.5 cm.	10 cm.
1		43,600	9,560	3,200	—	—	—	—	—	—
2		76,000	9,680	2,000	—	—	—	—	—	—
3		217,000	11,000	—	—	—	—	—	—	—
4		236,500	16,000	7,200	2,700,000	32,200	28,800	4.50	1.77	1.90
5		134,600	24,300	9,500	1,160,000	54,700	47,700	7.50	2.46	2.85
6		248,000	44,300	15,500	8,420,000	79,300	60,000	20.00	3.10	2.76

occasionally agar-decomposing forms. This surface layer of mud, having a high concentration of available organic matter and a source of available oxygen in the water above, appears to be the seat of the greatest activity of the marine bacteria. The number of bacteria in the surface mud varied markedly at the two sampling periods. However, the number of bacteria decreased on the ocean side of Cape Cod and increased correspondingly in the bay. It should be noted that, as shown in Table IV, the number of bacteria in the water of Cape Cod Bay also was markedly greater at this time than at the previous sampling period. It is generally assumed that there is an accumulation of sediment going on in Cape Cod Bay, part of this coming from the outside of Cape Cod. Whether there is any relation between this deposition of fresh sediment and the observed change in the bacterial content of the mud is uncertain. The fluctuation of the bacterial

content of the lower layers of mud is less marked. In Cape Cod Bay it was parallel with that of the surface mud, while off Cape Cod Light it was in the opposite direction. In the samples from the latter line there was again a progressive diminution in numbers of bacteria in the surface mud with distance from land. The explanation of this is not clear from the data available. In these samples the number of bacteria in the deeper layers did not decrease with distance from land. The vertical and horizontal distribution of bacteria in the mud of the Cape Cod Light line of stations is illustrated in Fig. 2. Because of the ex-

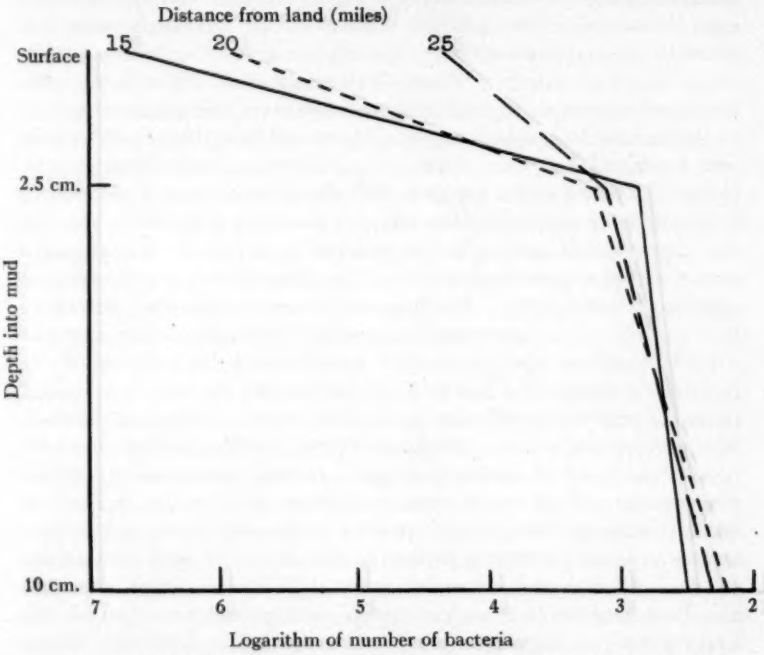

FIG. 2. Vertical and horizontal distribution of bacteria in the mud of the Atlantic Ocean off Cape Cod Light, August 24, 1932. —— Station 6; - - - Station 7; — — Station 8.

tremely wide range in the number of bacteria found, the logarithms of the numbers of bacteria are plotted, instead of the actual numbers themselves.

The relation between the bacterial content and the carbon content in the muds is very interesting. In the surface mud there does not appear to be a definite relation between the bacterial numbers and the

amount of carbon. This is to be expected, since the number of bacteria in this layer is not only due to the abundance of organic matter, but perhaps to a greater extent to its relatively undecomposed nature as compared to that in the lower layers of mud. This dependence of the organisms upon the nature of the organic matter is illustrated also by the vertical distribution of bacteria and carbon; the number of bacteria invariably decreased with successive increases in depth in the mud, while the carbon content often rose slightly. Oceanic bottom deposits are built up principally by the accumulation of new material at the surface. The rate of deposition is extremely slow and consequently mud at relatively short depths into the mud represents sediments laid down many years before. The organic matter in the mud below the surface has undergone extensive decomposition and only the more resistant substances remain. These substances are probably similar to the resistant organic complexes in the soil described by Waksman and Iyer (1932) and are higher in carbon than is the fresh organic matter. These factors, together with the practical absence of oxygen from the mud (Moore, 1931), offer an adequate explanation for the decrease of bacterial numbers with depth in the mud. The resistant nature of the organic matter limits the number of specific organisms capable of attacking it. The high carbon and low oxygen content of the organic matter decrease its availability to anaerobic bacteria, while the absence of oxygen in the mud limits the activity of aerobic forms. At depths of 2.5 and 10 cm. in the mud the horizontal distribution of bacteria varied directly with the carbon content of the mud. This was especially true of the mud in Cape Cod Bay, where the variation in the two factors was so large as to lend greater weight to the observations. This would seem to indicate that in the terrigenous deposits examined the organic matter in the mud below the surface layer was equally available to the bacteria at all the stations sampled and probably was similar in composition. The factors determining the distribution of bacteria at the surface of the mud are still obscure. Undoubtedly an important one is the stage of decomposition of the organic matter at the time it reaches the bottom.

Summary

A study has been made of the number of bacteria to be found in ocean waters and muds in the region of Cape Cod, Massachusetts, and their variation with distance from shore, depth of water, depth in the mud, and carbon content of the mud.

The number of bacteria in ocean water not near land and of a depth sufficient to preclude the agitation of the bottom by currents or wave

motion was very small. One cubic centimeter portions of the water were often found to contain no bacteria capable of growing on an agar plate. A few samples of water were found to contain comparatively large numbers of bacteria, probably due to the presence of decomposing remains of some small plankton organism.

Sea water in shallow regions where the water was mixed from top to bottom, due to tidal or wave motions, had a consistently higher bacterial content, ranging from 20 to 200 cells per cubic centimeter. Such water had a higher content of mineral nutrients and consequently a richer plankton content. It is to the larger amount of organic matter thus made available to the bacteria together with an actual transport upward of cells by vertical currents that their increased abundance under these conditions is to be attributed.

On open, exposed shores receiving little land drainage, the direct effect of the land on the bacterial content of the sea water appears to be negligible. Along shores receiving considerable land drainage any effect of the proximity of land does not appear to extend beyond a mile from the shore so far as the number of bacteria in the sea is concerned. In harbors receiving sewage drainage, the number of bacteria may rise to very high levels, depending upon the amount of pollution introduced and the degree to which circulation may take place between the harbor water and the outer uncontaminated water.

Bacteria in the bottom deposits were much more numerous than in the overlying water. Muds contained many more bacteria than did sands. The number of bacteria decreased with depth into the mud, the most marked decrease being in the first 2.5 centimeters. Bacterial numbers in the surface layer, compared to those in the lower layers, were much greater than could be accounted for by the difference in organic carbon content. There appeared to be on the surface of the mud a very thin layer containing organic matter in a less advanced stage of decomposition supporting a bacterial growth much richer not only in numbers but also in types of bacteria than in the lower layers. The carbon content of the surface layer was higher than that of the lower layers. The vertical distribution of bacteria in the mud was not related to the carbon content, since the amount of carbon sometimes increased with depth, while the number of bacteria always decreased with depth.

The horizontal distribution of bacteria in the extreme surface layer of mud was independent of its carbon content, but probably depended upon its composition or the degree of its decomposition. The horizontal distribution of bacteria in the mud layers below the surface was directly related to their carbon content.

The number of bacteria in the surface mud on the continental shelf east of Cape Cod decreased regularly with distance from land. This was not the case with the bacteria in the deeper layers of mud, where the number remained fairly constant.

There was a wide variation in the bacterial content of all layers of mud between two sampling dates approximately one month apart. The fluctuations at the surface and at depths of 2.5 and 10 cm. in the mud were in opposite directions in mud from the open ocean off Cape Cod Light but were parallel in mud from Cape Cod Bay.

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THE STRUCTURE AND DIVISION OF BODOPSIS GODBOLDI, SPEC. NOV.[1]

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MATERIAL AND METHODS

The organism for which the species name *godboldi* is proposed is a small flagellate protozoön of the order Parastomatida (Calkins, 1926). The genus contains one other species, *alternans*, described by Lemmermann (1914). The writer is unaware of any cytological work or any descriptive work on the genus other than that of Lemmermann.

The form herein alluded to developed in large numbers from a culture of raw sewage from the Plainfield, New Jersey, sewage disposal plant. A 1 cc. sample of sewage was inoculated into a Petri dish containing 10 cc. of the wheat medium used frequently by the writer (Lackey, 1927). Out of ten cultures made up at the same time this organism appeared in but one, but several subcultures were made in which the animals multiplied abundantly. Each culture was about 5 mm. deep and had a film of paraffin oil on top so that as the bacteria and protozoa multiplied the medium was at least partially anaerobic. Neither percentage of dissolved oxygen nor pH were ascertained.

Cover-glasses with films containing the living organisms were sealed to slides with lanolin for studying the activities of the animals. Prepared slides were made by sinking cover-glasses in the cultures until the organisms crept onto them, then fixing the film with either Gilson's fluid or 95 per cent alcohol saturated with $HgCl_2$ to which .5 per cent acetic acid was added. The first fixative proved excellent for the pseudopodia, which are fine and tenuous, while the second was best for the study of mitosis. Iron hæmatoxylin was the stain most frequently used, occasionally followed by eosin.

STRUCTURE OF THE ANIMAL

The living animal is generally ovate in outline and dorsiventrally bowed (Figs. 1, 2), but there is considerable deviation from this type shape. Individuals have a fixed shape, no amœboid changes having

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been observed. The average dimensions are 12 microns long, 6 wide, and 3 to 4 thick. This species is therefore slightly smaller than *B. alternans*. The pellicle is thin and unmarked. At the anterior end is a naked bowl-shaped depression, referred to as the mouth region. This is not at all like the gullet-reservoir system of the Euglenida. The two flagella emerge from this region and also the pseudopodia, when they are extended. Through the region food, including bacteria, is ingested. There is a contractile vacuole adjacent to this depression which empties into it. The flagella are dissimilar: the short one is about one-fourth body length, extends forward, and is vibratile. It is so short as to appear practically useless as far as locomotion is concerned. The longer is a trailing flagellum, half as long again as the body, and is held ventrally when the animal creeps or glides along the substratum.

The animal moves forward very slowly, perhaps making use of a creeping movement as well as the traction of the short flagellum. Often it is motionless for a long time, then moves slightly with a jerky motion. It was this very slow and jerky motion of a very large number of small animals on the bottom of a Petri dish which first called the organism to the attention of the writer. The motionless periods represent the times when the pseudopodia are extended and the animal is probably feeding. It has not been observed swimming, which might be impossible with its very short flagellum. In *B. alternans* the two flagella appear to be of equal length and the difference in this respect is the most marked one between the two species.

The cytoplasm is hyaline and homogenous in appearance. Food vacuoles, at times containing bacteria and portions of small fungi as recognizable contents, and moving with a slow cyclosis, are often seen. In the posterior part of the cell a number of small bright spheres of unknown nature are usually present. The nuclear membrane with a clear zone just inside it and a heavy endosome are visible in the living animal.

The pseudopodia (Fig. 3) are best studied in fixed, overstained animals. From two to six in number, they emerge from the mouth region and extend laterally from the ventral surface as a rule. The cytoplasm at such times may flow out to partly cover an area of the pellicle, much as it extrudes from the mouth of the shell of an *Arcella*. The pseudopodia are very fine, of the filose type, and branch infrequently. Often there are small enlargements, blobs of protoplasm on them, and it has been definitely observed that they are used in the taking of bacteria. Where they come in contact or cross, they do not anastomose. Their greatest extension rarely exceeds twice body length. There is no axial filament.

The kinetic elements are a pair of blepharoplasts (Figs. 1, 4) adjacent to the nuclear membrane and in the thin layer of cytoplasm which is the floor of the mouth depression. It is very difficult to determine that there are two, so small and so close together are they. The nucleus is in the anterior part of the body and appears to be slightly

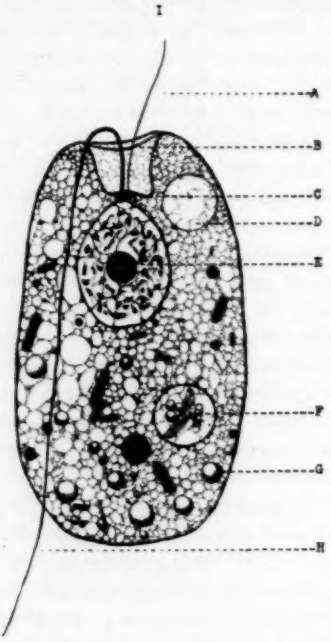

FIG. 1. *Bodopsis godboldi*, spec. nov. Greatly enlarged to show structure. Nuclear and kinetic structures drawn from prepared slide, cytoplasmic detail from a study of living animals. *A*—anteriorly directed flagellum. *B*—mouth depression. *C*—blepharoplasts. *D*—contractile vacuole. *E*—endosome, surrounded by the chromatin masses characteristic of the vegetative nucleus. *F*—food vacuole. *G*—cytoplasmic sphere, one of three common types of inclusions. Two others are shown, but not labeled: the black spheres, visible only after staining, and the black rods also visible only after staining. *H*—trailing or gliding flagellum.

pointed or pulled up to make contact with the blepharoplasts. Its membrane is rather heavy. There is always a central round endosome, with a small clear zone between it and the chromatin which instead of being in the form of granules appears to have the form of a permanent, faintly staining spireme. This detail is not to be ascertained with certainty in the interkinetic stages.

Cell Division

The animal apparently does not reach a maximum size before division, since both large and small ones are found dividing. Division is longitudinal in the active state, the nuclear membrane persisting throughout the process. The first indication of division is the increase in the staining capacity of the chromatin, which soon forms a number of heavy, irregular masses of chromatin, at first assumed to be comparable to the prochromosomes of some of the higher organisms (Fig. 4). Attempts to count the number of these have not been successful, partly because of their irregular contours and partly because of the presence of the endosome. They tend to assume a more or less peripheral position inside the nuclear membrane, and a number of counts have been made which show more than thirty of them. We have concluded after studying this particular phase of division that there are more than the metaphase number of chromosomes and less than twice the number, and that the number of these irregular chromatin masses is not constant.

As these masses are forming, the endosome begins to lose its power of taking the stain. It finally disappears altogether, simply becoming faint, then vanishing altogether, before the metaphase plate of chromosomes is formed. It does not contribute to the formation of blepharoplasts or new flagella, nor visibly to the formation of the chromosomes. It is possible that it is of chromatin as Belar (1920) has shown to be the condition relative to the endosome of *Prowazekia*.

By the time the endosome has disappeared, the chromosomes are fully formed. There are 24 of these and they are small round bodies, not connected with each other, and usually close to each other when they have oriented themselves on the intranuclear spindle. The origin of the spindle is impossible to trace; it first becomes visible at the time the metaphase plate of chromosomes is formed. Because of its small size it is impossible to distinguish spindle fibers, but the double cone shape and the orientation of the metaphase plate of chromosomes on it leaves no doubt as to its identity. There are no centrioles at its poles, but its ends reach to the nuclear membrane at this time (Fig. 6). It is impossible to say whether the ends of it still reach the membrane in the anaphase and telophase stages (Figs. 8, 9) or not, but spindle remains, presumably mantle fibers, are present in these stages. The spindle invariably occupies a position at right angles to the long axis of the cell, and the metaphase plate of chromosomes is therefore always in equatorial view, since animals are rarely found turned edgewise to the observer. For this reason it is difficult to find plates on which counts of the chromosome number can be made.

The chromosomes practically all divide at the same time, assuming a dumb-bell shape (Fig. 6*b*), then parting. There are none which lag on the spindle, and due to the fact that they are all approximately round in the beginning, no question of their plane of division can be discussed.

During these happenings the original pair of flagella has persisted,

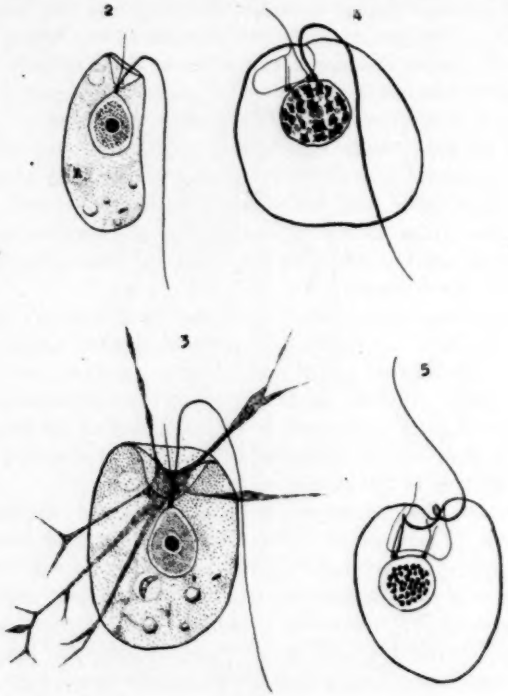

PLATE I

FIG. 2. Side view of the animal to show characteristic convex shape.

FIG. 3. Ventral view of an animal to show pseudopodia which are extended from the mass of cytoplasm extending out of and over the ventral lip of the mouth depression.

FIG. 4. Early prophase. The chromatin has formed into a number of irregular masses, apparently separate from each other and not forming a spireme. The endosome is disappearing. Two daughter blepharoplasts are present, and two new and short flagella are growing out from them through the mouth depression. This figure and the remaining ones are optical sections through the nucleus, but the flagella have been added to the sections.

FIG. 5. Later prophase. The endosome has disappeared and the chromatin masses are more condensed. The new blepharoplasts and flagella are clearly seen.

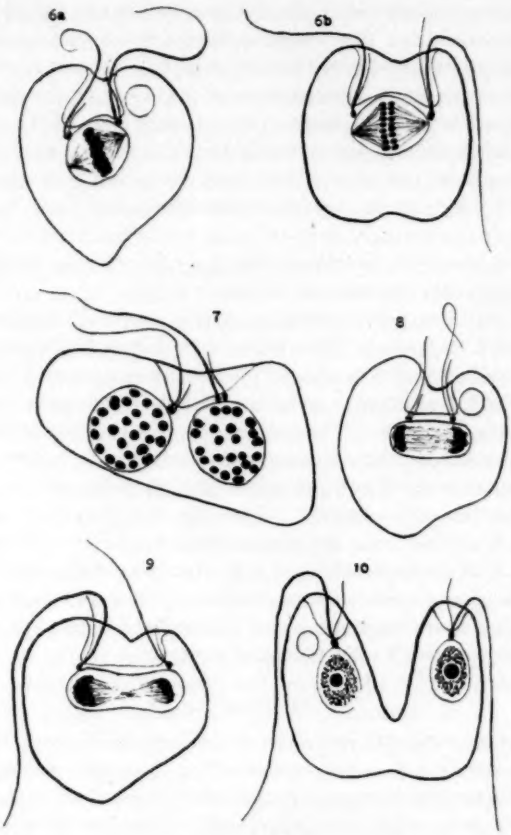

PLATE II

FIG. 6*a*. Metaphase plate showing the equatorial mass of chromosomes, the intranuclear spindle, and the blepharoplasts and flagella.

FIG. 6*b*. Metaphase plate showing the dividing chromosomes.

FIG. 7. Anaphase. This figure is slightly diagrammatic in that the two groups of chromosomes have been actually shifted slightly to the right and left. In the animal studied they overlap each other about one-third but are so far apart that the two optical sections each give a clear count of the chromosomes. Each group consists of 24 chromosomes.

FIG. 8. Telophase. The chromosomes have reached the poles of the spindle. Clearly distinguishable spindle remains are seen.

FIG. 9. Telophase. Initial constriction of the cell and of the nucleus.

FIG. 10. Telophase. Nuclear reorganization practically complete and the two daughter cells about ready to separate.

but has come to occupy an eccentric position in the mouth depression which has widened. The blepharoplasts are near one pole of the mitotic figure, but do not form the pole (Fig. 6). During the prophases a second set of blepharoplasts appears at first close to the old ones, then moving apart from them toward the opposite pole of the spindle. It has been impossible to say certainly that this second pair of blepharoplasts arises from the original pair by division, but their position when first discernible, and their gradual migration away from the persistent original pair are strongly in favor of an origin by division, and against an origin *de novo*. It is certain that they do not arise from the endosome or any other nuclear component.

From this new pair of blepharoplasts, two new flagella grow out (Figs. 4 to 8 inclusive). The initial size difference between the two pairs is readily apparent, and precludes any question of the origin of new flagella by splitting: one of the daughter cells gets the old pair, the other the new pair. The animal is active throughout division.

In the anaphase the chromosomes move apart rapidly. Figure 7 shows polar views of the two daughter groups of chromosomes in which the number may be counted. The chromosomes become indistinguishably mingled in the telophase (Figs. 8 and 9) and still stain so deeply that it is impossible to tell when the endosome reappears. About this time a median anterior furrow begins to constrict the cell, dividing the mouth depression, and the nuclear membrane also begins to constrict, the cell finally pinching in two.

Discussion

The Pantastomatida represent a very primitive group of protozoa and in the ten or twelve genera comprising this order we might reasonably expect to find primitive modes of reproduction. Such a condition has been described by Goldschmidt (1904, 1907) in the case of *Mastigella* and *Mastigina*, where the chromatin granules pass through the nuclear membrane and after accumulating on the outside, reform into the nuclei of gametes. Doflein (1916 *a* and *b*) has criticised the account of Goldschmidt, but has not shown him to be wrong. On the other hand, Minchin (1912) says that such a process would represent a step in the evolution of the protozoa from organisms such as bacterial types.

Accounts of division of other organisms of this order are lacking. It is therefore interesting to find in *Bodopsis*, representative of the order, a type of division not comparable to reproduction as described by Goldschmidt for *Mastigella* and *Mastigina* but more like that described by Wenyon (1926) for *Cercomonas*, Hartmann and Chagas

(1910) for *Spongomonas*, Belar (1920, 1921) for *Bodo edax* and *Collodictyon*, or even some of the Phytomonadida as shown by Aragao (1910), Doflein (1916, *a* and *b*) and Kater (1925). The pseudopodia of *Bodopsis* clearly place it in the Pantastomatida, but its type of nuclear division brings it into a more closely conformed relationship with these protozoa of related orders. Gamete formation in the manner described by Goldschmidt is not precluded, however, since gamete formation might occur at some stage in the life history not covered by this account.

It is noted that the nuclear membrane is persistent throughout division. This is not new, as shown in *Cercomonas*, *Spongomonas*, and *Cyathomonas* by Hartmann and Chagas, *Polytoma* by Kater, *Peranema* and *Entosiphon* by Lackey (1929, 1933), and *Euglena* by Baker (1926) and others, as well as various other protozoa. In *Bodopsis*, however, the chromatin lies just beneath the nuclear membrane as if the nucleus is turgid with a fluid pressing the masses of chromatin against the membrane. But no diffusion out or passage out of chromatic material can be noted. Instead the whole mechanism of chromosome formation and division is intranuclear even including the spindle. It is a fascinating idea that the endosome might give rise to the spindle. Thus in *Bodopsis*, *Cercomonas*, *Spongomonas*, *Cyathomonas* and other protozoa where there is an endosome which completely disappears during division, a well-defined spindle is formed which is an intranuclear structure in the four above-listed forms. On the other hand, in *Entosiphon*, *Peranema*, *Euglena* and others where the endosome does not disappear during division, no spindle is formed.

A peculiarity of *Bodopsis* is that while no centrioles are present, the blepharoplasts do not serve as division centers. It has been repeatedly stated recently that it is a difficult matter to determine just what is a centriole, but certainly there are no distinguishable ones in *Bodopsis*, whereas in most of the flagellates whose division has been carefully studied, either centrioles or blepharoplasts serve as poles of the division figure, the relations between these having been admirably diagrammed by Minchin (1912, p. 88). Centrioles have been figured for *Polytomella*, *Polytoma*, *Collodictyon*, *Bodo*, *Spongomonas splendida* and *S. uvella*, *Astasia* and others. Blepharoplasts serve as division centers in *Cercomonas*, *Peranema*, *Entosiphon*, and *Menoidium* (Hall, 1923). Since neither of these serves as a division center in *Bodopsis*, the question arises as to what actually constitutes the division center; or if there is one. The apparent absence of one is a condition unique for the flagellate protozoa.

If the primitive living cell has a distributed nucleus, it is hardly

to be expected that the flagella of such a cell will possess blepharoplasts and the writer knows of no mention of them by bacteriologists. On the other hand, the possession of blepharoplasts by *Bodopsis* in juxtaposition to a well-organized nucleus, even if they do not serve as division centers, would argue that it is a specialized, not a primitive organism. This is further borne out by its mitotic type of nuclear division. And unless there is some other stage in its life history in which phenomena occur more comparable to those described by Goldschmidt for *Mastigina*, it is perhaps wiser to base the concept of the primitive condition of the order on the possession of both flagella and an amœboid body with pseudopodia.

Conclusions

1. *Bodopsis godboldi*, spec. nov., is shown to have a very short anteriorly directed flagellum, this being the chief character in which the species differs from *B. alternans*.

2. Its nuclear division is accomplished in the active state and is followed by binary fission.

3. The endosome disappears and the chromatin forms 24 small round chromosomes on an intranuclear spindle which has neither centrioles nor blepharoplasts for poles.

4. The original blepharoplasts and flagella are retained. New blepharoplasts apparently arise from the old ones by division, and new flagella from the new blepharoplasts by outgrowth.

5. The blepharoplasts occupy eccentric positions on the nuclear membrane while mitosis is occurring but do not form the spindle poles. It is concluded they are not the division centers.

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ON A NEW GREGARINE FROM THE CŒLOM OF A BURMESE EARTHWORM, PHERETIMA COMPTA

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In certain oriental species of earthworms, especially of the genera *Eutyphœus* and *Pheretima*, gregarines (trophozoite stages) are very frequently found in the cœlom. These gregarines when present may occur in large numbers, 50–90 per segment for a number of segments. Among the forms that have come under observation during the course of a study of Burmese earthworms is one which appears to be of especial interest and which is described below under the designation *Nellocystis birmanica, n.g., n. sp.*

Hitherto it has been assumed that the effect of the gregarine parasites on their earthworm hosts is not serious. Indeed, Stephenson in his monograph on the Oligochætes says, "The intestinal and cœlomic parasites have scarcely any effect on the tissues of the host." There is, however, some evidence to show that cœlomic sporozoa may have a drastic effect on host earthworms. A part of this evidence is included in a paper on the systematics of the Burmese earthworms that will appear shortly in the Records of the Indian Museum. It is hoped that the rather fragmentary observations contained in the paper will serve to indicate to protozoölogists a number of interesting opportunities for investigation.

NELLOCYSTIS BIRMANICA, N. G., N. SP.

The parasites are present as solitary individuals or aggregates of two or three individuals.

Solitary Individuals

The exact length of the parasites was not determined as the individuals were broken in removing them from the host for study. The form may be described as club-shaped, the posterior end enlarged, narrowing to an elongate stalk. The anterior end of the stalk is branched into several short filaments which attach the parasite to the host. In Fig. 2 is shown a portion of the anterior end of a parasite as it appears after being pulled out with forceps from a very delicate intersegmental septum.

In the fixed condition the stalk and a thin layer of the posterior end

is solid and can be cut up into fragments which do not lose their shape.

Within the posterior portion of the parasite is a cavity, referred to as the vacuolar cavity, containing a fluid in which there is a single, dark, regularly spheroidal mass. The mass nearly fills the cavity and when ruptured in water or alcohol separates into granules which are connected with each other by fine cobwebby strands. Within the granular mass is a single, ovoidal nucleus. This is of a clear, transparent blueish or yellowish appearance and contains a single endosome. Within the stalk near the vacuolar cavity is a second nucleus which is like the vacuolar nucleus but which contains in addition to a large endosome, a smaller, slightly bent, rod-like body. In some of the indi-

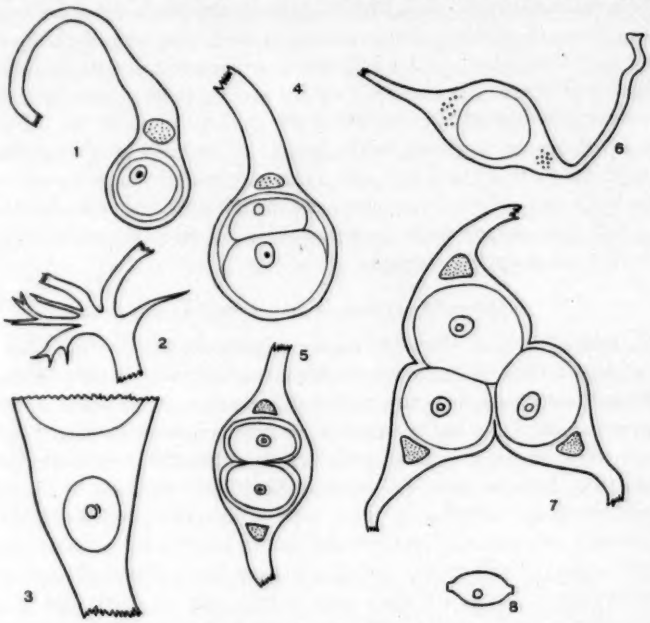

FIG. 1. Portion of a solitary individual, × 50.
FIG. 2. Portion of an anterior end of a solitary individual, × 160.
FIG. 3. Portion of a solitary individual showing the stalk nucleus in longitudinal position, × 160.
FIG. 4. Posterior end of a solitary individual containing two vacuolar masses, × 50.
FIG. 5. Central portion of a bi-stalked form with vacuolar septum, × 50.
FIG. 6. Bi-stalked form in which the vacuolar cavity was filled with cysts, × 50.
FIG. 7. Tri-stalked form, × 50.
FIG. 8. Cyst, × 330.

viduals the long axis of the stalk nucleus is along the long axis of the stalk (Fig. 3). In other individuals the major axis of the stalk nucleus is transverse to the long axis of the stalk (Fig. 1). In all those specimens in which the stalk nucleus is in the transverse position the single, large endosome is replaced by a number of smaller, endosomal granules scattered throughout the nucleus.

Several individuals were found in which the single vacuolar cavity contains two discrete, ovoidal masses, in each case one of the masses larger than the other. Each of these two masses has a single nucleus with an endosome. In specimens with two vacuolar masses the stalk nucleus is not ovoidal but the side of the nucleus towards the vacuolar cavity is flattened as shown in the figure. The endosomal material of the stalk nuclei is in the form of granules scattered through the nucleus. A few individuals differ from those just described in the replacement of the single nucleus of each vacuolar mass by several smaller bodies apparently of a nuclear nature.

Aggregates of Two or Three Individuals

Bi-stalked Forms.—These forms are spindle-shaped and have a stalk at each end so that the animal is doubly attached to the host. Stalks of all of these were broken in the course of dissection of the worm or in removing the parasites and the ends were not recovered so that it is not known whether the attaching ends have branched filaments similar to those at the anterior ends of solitary individuals. In some of these bi-stalked forms the vacuolar cavity is divided into two portions by a thin but distinct septum, each half of the cavity containing a single, dark, ovoidal mass. Each of the vacuolar masses has a single nucleus with an endosome. There are two stalk nuclei, one at each side of the partitioned vacuolar cavity, the side of the nucleus towards the cavity always flattened. In other bi-stalked forms the vacuolar septum is lacking and within the vacuolar cavity there is a single, dark mass. No nucleus is at first visible within the mass but if the mass is ruptured and flattened out carefully there may be seen among the granules small, ovoidal bodies similar in appearance to an endosome but smaller and with a cobwebby material attached to the surface so that each has a rather fuzzy appearance. In some specimens the number of these bodies is rather small; varying apparently from 16–32. In other specimens the number is much larger.

In a few bi-stalked forms the vacuolar cavity is filled with cysts. No definite stalk nuclei were observed in cyst-containing forms but at the approximate site of each nucleus there is an aggregation of endosomal-like granules. The regions between and around the endosomal

granules are occupied by stalk substance rather than the clear and transparent extra-endosomal nuclear material.

The cysts are pseudonavicella-like (homopolar). There is a single nucleus, eccentrically located within the finely granular contents. One of the cysts is shown in Fig. 8.

Tri-stalked Forms.—A number of tri-stalked forms were found, aggregates of three individuals, each individual with a nucleated vacuolar mass and a stalk nucleus flattened on the vacuolar side and containing numerous endosomal granules. Attaching structures at ends of the stalks were not found.

Occurrence

Attached to the cœlomic face of the body wall, to the gut, and to the septa, in the region of segments viii–xxv of the type specimen of *P. compta* Gates 1932. The host was secured in the jungle near Blachi on the Karen Hills (western border of the Shan Plateau) to the east of Toungoo, Province of Burma, in the month of September.

Remarks

Although the host specimen was collected and preserved under unfavourable conditions the parasites are well preserved so that the various structures can be easily made out without either clearing or staining, the specimens having been examined as a rule only in the fluid used for preservation of the host. The figures are camera lucida sketches made immediately after removal of the parasites and prior to staining and clearing.

SUMMARY

A new species of gregarine protozoan, *Nellocystis birmanica*, elongately club-shaped, attached to the host by short branches of the anterior end, with two nuclei, parasitic in the cœlom of an earthworm, *P. compta*, from the Toungoo Hills of Burma, is described.

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OSMOTIC PROPERTIES OF THE ERYTHROCYTE

VI. The Influence of the Escape of Salts on Hemolysis by Hypotonic Solutions

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I

In its osmotic behavior the erythrocyte shows certain anomalies that have never been completely explained. For example, it is known to undergo considerably less swelling in hypotonic solutions than it would if it were a perfect osmometer. This is particularly true of hypotonic solutions of non-electrolytes. A part of the discrepancy between the observed and the theoretical behavior of the cell is undoubtedly due to the fairly large fraction of its volume occupied by hemoglobin, and, to a much less extent, by other protein molecules; indeed, according to Ege (1922), when an entirely reasonable allowance of 35 or 40 per cent of the cell volume is made for this "osmotic dead space," the discrepancy largely vanishes. In the opinion of other workers, however (Gough, 1924; Krevisky, 1930; Ponder and Saslow, 1930), the difference between the observed and the theoretical volume changes is too great to be accounted for in this way; according to them the apparent dead space may amount to as much as 65 or 70 per cent of the total volume. Whatever may be the truth about this disputed point, it is certain that some other factor must be involved in producing the exceptionally low volumes observed in non-electrolyte solutions.

Two chief explanations have been offered to account for the above-mentioned peculiarities of the erythrocyte. The first (Gough, 1924; Krevisky, 1930; Ponder and Saslow, 1930) is that a part of the water in the cell is "bound" in such a way that it is unable to take part in osmotic exchanges. This explanation is not in accord with the results of Hill (1930), whose vapor pressure measurements indicate that no considerable part of the water of the erythrocyte fails to make its proper contribution to the colligative properties of the cellular solutions. For this reason, Ponder and his collaborators (Ponder and Saslow, 1931; Macleod and Ponder, 1933) have more recently strongly supported a second explanation, namely, that the failure of the ery-

throcyte to swell to the expected extent is due to a rapid escape of osmotically active materials from the cell into such solutions, particularly into those of non-electrolytes. In favor of this theory, in addition to their own important evidence, they cite the demonstration by Kerr (1929) that the erythrocytes of several mammals lose their normal impermeability to cations in protein-free solutions. The work of Joel (1915) on the progressive increase in the electrical conductance of suspensions of erythrocytes in solutions of sucrose is probably also to be interpreted in the same way, and numerous other observations scattered through the literature add further evidence in the same direction.

In view of all the available information, it can scarcely be doubted that under appropriate conditions an escape of salts from the erythrocyte into various non-physiological solutions takes place; and such an escape may well account for some of the osmotic peculiarities of this type of cell. Additional evidence of its occurrence will be given in the present paper. We believe, however, as a result of the observations about to be described, and of others as yet unpublished, that in experiments of short duration a leakage of osmotically active materials from the erythrocyte is a factor of relatively minor importance. In particular, we believe that the peculiar osmotic behavior of the erythrocyte in solutions of non-electrolytes, which has been the cause of much speculation since it was first observed by Hamburger, can to a considerable extent be accounted for in an entirely different manner, without postulating any loss of the normal impermeability of the cell to cations and without invoking factors other than those generally agreed to operate under typical physiological conditions.

II

The method we have employed is based upon a very briefly recorded observation of Green (1925), who reported that human erythrocytes, which ordinarily always undergo hemolysis in a 0.3 per cent solution of NaCl, may fail to do so after a preliminary treatment with non-hemolytic hypotonic solutions. The most plausible explanation of this behavior, which is the one proposed by Green, is that during the initial exposure to the non-hemolytic solution the swollen cells lose salts, and having thus acquired a lower internal osmotic pressure, they swell less than would otherwise be expected when the external concentration is further lowered. In this way they may escape hemolysis in hypotonic solutions in which it would ordinarily occur.

Unfortunately Green mentions no times in his account of his experiments, so it is impossible to be certain from his description whether the phenomenon he observed occurs rapidly or slowly. Furthermore,

his method of comparing the osmotic resistance of the treated and the untreated cells is a very rough one. It seemed advisable, therefore, to take up the problem again in a more accurately quantitative manner. This we have done, employing the method described in the first paper of this series (Jacobs, 1930). By its use it is possible to measure percentages of hemolysis with a highly satisfactory degree of reproducibility, provided that the following precautions be used: (1) accurate measurement of the blood, (2) maintenance of a constant temperature, (3) maintenance of a constant pH, and (4) slow but constant stirring throughout the experiment. These precautions we have observed. In particular the pH of all our NaCl solutions was adjusted in the manner more fully described in the second paper of the series (Jacobs and Parpart, 1931) to approximately the normal blood reaction, and the temperatures were kept within a few tenths of a degree of those recorded in the tables.

TABLE I

Ox erythrocytes exposed for varying times to 0.110 M NaCl and then allowed to undergo hemolysis in 0.094 M NaCl. Temperature, 19.8° C.

Time of exposure	Final percentage of hemolysis
0	69
1 minute	69
10 minutes	69
30 minutes	68
1 hour	69
3 hours	68
5 hours	65
7 hours	62
9 hours	59
11 hours	56
18½ hours	54
21 hours	49

The general procedure followed in all the experiments may be made clear by outlining a typical one whose results are summarized in Table I. In this experiment, which was performed upon ox blood, preliminary determinations showed that 69 per cent of the erythrocytes underwent hemolysis in an hour in a 0.094 M solution of NaCl. This percentage showed little or no increase on further exposure, and could be obtained accurately and reproducibly in successive experiments on the same lot of blood. Different samples of blood were therefore exposed to a slightly more concentrated, non-hemolytic, but still strongly hypotonic (0.110 M) solution of NaCl and then, after times varying from one second to many hours, the concentration of this solution was suddenly reduced by dilution to 0.094 M. In order that hemolysis might, in all cases, take place with constant proportions of blood and of

the hemolytic solution, the standard degree of hemolysis was determined by adding two drops of blood to 50 cc. of 0.094 M NaCl, the initial exposure to 0.110 M NaCl was made by adding two drops to 25 cc. of solution, and the subsequent dilution was brought about by mixing with this suspension an equal volume of 0.078 M NaCl. In this way the proportions of blood and solution were kept exactly the same during the process of hemolysis in all the experiments and in the control, namely, two drops of blood to 50 cc. of solution.

It would be expected that if an escape of salts occurred in the manner postulated by Green during the preliminary exposure to the nonhemolytic hypotonic solution, a lower degree of hemolysis would be found after this treatment than in the control. Since the method of measurement we employed is a delicate one, giving distinct differences in hemolysis with osmotic pressure, differences of a fraction of one per cent of that of blood plasma, any considerable loss of salts could scarcely have escaped detection. Nevertheless, as seen in Table I, the expected increase in the osmotic resistance of the cells began to be visible only after 3 to 5 hours, and its subsequent progress, though steady, was decidedly slow. The same general result was obtained in seven successive experiments with ox blood without a single exception. It seems likely, therefore, that with this particular kind of blood, which was used almost exclusively in our earlier work on osmotic hemolysis, an escape of salts in the course of experiments whose individual duration was usually from a few seconds to an hour at most could scarcely have been a disturbing factor of much significance. The criticism of this work by Macleod and Ponder (1933) on the grounds that we neglected the escape of salts from our cells, though theoretically an entirely valid one, would seem practically to be no more serious than similar criticisms that might be made of any of the simplifying assumptions that entered into our admittedly preliminary and approximate calculations.

It might perhaps be thought that in the experiment just described the initial degree of swelling of the cells was insufficient to permit a ready escape of salts but that a greater degree might have done so. To meet this objection, experiments were carried out in which a preliminary exposure was made to a solution which caused a considerable amount of hemolysis. In this case, the cells that just escaped hemolysis might be expected to show the optimum conditions for the escape of salts; nevertheless, although our method was capable of detecting osmotic changes of the expected sort in any group of more than one or two per cent of the cells used, such changes consistently failed to appear with ox erythrocytes in times less than several hours. We may

conclude, therefore, that even under the most extreme conditions of swelling possible without hemolysis, the escape of salts from these cells must be fairly slow.

Another possible objection is that perhaps the escape of salts occurs so rapidly that even in the control it is complete before hemolysis has had time to take place, and for this reason differences between the experiment and the control fail to appear. In answer to this objection, which evidently cannot be completely met by experiments on hemolysis alone, it may be said that any escape of materials from the erythrocyte that proceeds as rapidly to a fixed end-point as the one just postulated can scarcely be the same as those studied by Kerr and by Joel, which apparently take place gradually and progressively over a period of hours. It is also difficult to believe that it is the same as the one indicated after 5 hours in Table I, since it is highly unlikely that a mere diffusion process would proceed almost instantly to some fixed point, then cease for several hours, only to be resumed again at the end of that time. If a rapid, indeed apparently an almost instantaneous, change in the osmotic properties of the erythrocyte occurs on placing it in a hypotonic solution—which is by no means inconceivable—it is probably due to some other cause than a mere outward diffusion of salts. The extreme definiteness of the behavior of erythrocytes in hypotonic solutions, which is brought out very clearly by the work of Ponder and Saslow (1931), suggests the possibility of a changed ionic equilibrium of some sort rather than a mere leakage of salts. In the case of non-electrolyte solutions, we believe that a plausible suggestion can be made as to the possible nature of such an ionic equilibrium, and this will be given below; in the case of electrolyte solutions, however, we have at present no definite explanation to offer.

III

The experiments so far described were performed upon the erythrocytes of the ox, which had been used in most of our previously published work. After confirming our earlier impression that the hemolytic behavior of these cells is probably not complicated to a troublesome extent in experiments of moderate duration by a leakage of salts, we examined in the same way the erythrocytes of several other species of mammals. The result of these experiments was to show specific differences of a rather surprising magnitude. Unlike the erythrocytes of the ox, which remained apparently unchanged in non-hemolytic hypotonic solutions for a number of hours, those of the pig, cat, rabbit and, in the single experiment as yet made, those of man usually showed fairly marked changes within an hour, and in some

cases within a half-hour or less. A typical experiment with erythrocytes of the cat is summarized in Table II, and one with those of the rabbit in Table III. In the case of the cat cells, it will be noted that a decided increase in resistance was apparent in 30 minutes, and that roughly as much change had occurred in 3 hours as had been previously found with the ox cells in 21 hours. The erythrocytes of the pig seem to be somewhat more variable in their properties than those of the cat, the observed differences apparently depending to a considerable extent on the "age" of the cells. At the one extreme was a case where a marked increase in resistance occurred in 10 minutes and, at the other, one where no change was apparent in 2 hours.

TABLE II

Cat erythrocytes exposed for varying times to 0.100 M NaCl and then allowed to undergo hemolysis in 0.087 M NaCl. Temperature, 21.2° C.

Time of exposure	Final percentage of hemolysis
0	76
1 minute	75
10 minutes	74
30 minutes	70
50 minutes	69
70 minutes	68
90 minutes	66
2 hours	65
3 hours	59
4½ hours	59
7 hours	53

TABLE III

Rabbit erythrocytes exposed for varying times to 0.095 M NaCl and then allowed to undergo hemolysis in 0.072 M NaCl. Temperature, 22.7° C.

Time of exposure	Final percentage of hemolysis
0	58
1 minute	57
5 minutes	61
10 minutes	62
30 minutes	59
1 hour	51
2 hours	45
3 hours	36
4 hours	30
5 hours	28
8 hours	29

In the experiments on rabbit erythrocytes, a typical example of which is represented in Table III, the results were somewhat unexpected. The fall in the percentage of hemolysis which we have

interpreted as being due to an escape of salts occurred fairly rapidly, as with the cat, but unlike the cases previously described, this increase in resistance was preceded by a temporary fall. In other words, rabbit cells after short exposures to a non-hemolytic hypotonic solution seem to be slightly more easily hemolyzed than the controls, though after longer exposures they behave in the more typical reverse manner. Perhaps factors such as those discussed by Ege (1921) may be involved. In connection with the use of rabbit erythrocytes, for osmotic experiments, reference may also be made to the recent work of Csapó and Kerpel-Fronius (1933), who have pointed out that this species is remarkable among mammals in the large fluctuations it shows in the depression of the freezing-point of its blood. According to these authors the rabbit is able to maintain constancy of blood pH only at the expense of the constancy of osmotic pressure usually found in mammals. For this reason it would seem that until the properties of rabbit's blood have been very fully investigated, considerable caution should be used in extending to other species conclusions drawn from experiments on it alone.

From this brief summary of our results on the erythrocytes of several species of mammals other than the ox, it is evident that not all kinds of blood are equally suitable for osmotic experiments. To the extent that changes such as those indicated in Tables II and III occur, the escape of salts from swollen cells must be regarded as a disturbing factor in all such experiments. The magnitude of the resulting errors, however, is usually not so great as might be imagined, since the method here employed is a delicate one which brings out clearly small differences. For example, a change in the percentage of hemolysis from 76 per cent to 70 per cent, such as occurred in the erythrocytes of the cat in 30 minutes, corresponds to a concentration difference of the order of magnitude of 0.001 M NaCl, *i.e.*, to an osmotic pressure difference less than 1 per cent of that of the blood. While, therefore, the erythrocytes of the pig, the cat, and the rabbit are obviously less suitable for osmotic experiments than are those of the ox, which by a lucky accident we happen to have used in most of our previous work, even they may be expected in experiments of not too great duration to yield results that are not hopelessly complicated by an escape of salts.

IV

The apparently abnormal osmotic behavior of erythrocytes in solutions of non-electrolytes has been recognized since the early work of Hamburger (for a general summary, see Hamburger, 1902). The view that this behavior is due to an escape of salts was expressed by Bang

(1909) and has recently received additional support from Ponder and Saslow (1931). Our own experiments on hemolysis in hypotonic non-electrolyte solutions, made by the method already described, confirm the general conclusion that osmotically active substances escape with especial readiness into such solutions. They indicate, however, that there is an earlier and a more characteristic effect of non-electrolyte solutions of a very different nature, which is entirely compatible with, and indeed dependent on, the normal impermeability of the erythrocyte to cations. The escape of salts, *i.e.*, of both anions and cations, which undoubtedly occurs, seems to be a later and a more pathological phenomenon.

TABLE IV

Ox erythrocytes exposed for varying times to 0.2 M sucrose and then allowed to undergo hemolysis in 0.125 M sucrose. Temperature 20° C.

Time of exposure	Final percentage of hemolysis
0	61
1 second	56
5 seconds	52
10 seconds	35
20 seconds	24
30 seconds	23.5
1 minute	21.5
5 minutes	18.5
10 minutes	18.0
15 minutes	18.0
20 minutes	13.0
30 minutes	7.0
45 minutes	5.5
60 minutes	0

In Table IV are presented the results of a typical experiment on ox erythrocytes with a non-electrolyte solution. In striking contrast with the behavior of these cells in hypotonic non-hemolytic NaCl solutions in which their normal osmotic properties are maintained for hours is that in similar solutions of sucrose. In the present experiment it will be noted that as much change occurred in 1 second as in that previously described (Table I) in perhaps 5 hours. Within 20 seconds the change was greater than that ever obtained in a day with a hypotonic NaCl solution. Do such results indicate an equally great difference in the rate of escape of salts from the cells in the two solutions? We believe that they do not, but that the change that occurs in erythrocytes exposed to non-electrolyte solutions consists of two phases: a very rapid ionic shift, whose effects are apparent within a few seconds, and complete within a minute or two, and following this, usually after a distinct pause, which in the present experiment amounted

to approximately 10 or 15 minutes, a much slower leakage of salts from the cell, which is, nevertheless, considerably more rapid, and which begins much earlier than is the case with the similar change in electrolyte solutions (cf. Table I). As far as the second change is concerned, we are in agreement with Bang (1909) and with Ponder and Saslow (1931). We differ from them, however, in our interpretation of the first and more striking change which has apparently not previously been recognized as a different phenomenon.

Our reasons for considering the rapid initial change as due to an ionic exchange rather than to a leakage of salts are: first, its rapidity and definiteness; second, the fact that it occurs in isosmotic as well as in hypotonic sucrose solutions, whereas, according to Ponder and Saslow

TABLE V

Ox erythrocytes exposed to 0.2 M sucrose for 3 minutes and then for varying additional times to 0.2 M sucrose containing 0.002 M NaCl. Final hemolysis in 0.125 M sucrose or in same + 0.001 M NaCl. Temperature, 20° C.

Time of exposure	Final percentage of hemolysis
No initial exposure to 0.2 M sucrose	61
3 minutes exposure to 0.2 M sucrose	20
Same after the following further exposures to 0.2 M sucrose + 0.002 M NaCl	
30 seconds	53.5
1 minute	60
2 minutes	60.5
5 minutes	55.0
15 minutes	54.0
30 minutes	52.0
40 minutes	52.5
50 minutes	47.5
100 minutes	44.5
160 minutes	34.5
255 minutes	12.0

(1931), leakage of salts in general takes place only in the latter, in proportion to their degree of hypotonicity; and third, and most important, the fact that this effect is readily reversible while the second and slower one is not. A typical experiment showing this reversibility is summarized in Table V. As indicated in this table, an exposure of ox erythrocytes for 3 minutes to 0.2 M sucrose reduced the degree of hemolysis in 0.125 M sucrose from 61 per cent to 20 per cent. For a time this new level was maintained before falling further. If, however, the cells after a 3 minutes exposure to 0.2 M sucrose were placed in 0.2 M sucrose + 0.002 M NaCl for varying times and the resulting suspension was then suddenly diluted to 0.125 M sucrose + 0.001 M NaCl, the percentage of hemolysis returned to about its initial

value. The same general results were obtained when the final solution was 0.125 M sucrose + 0.002 M NaCl. In other experiments it was found that $CaCl_2$ is roughly twice as effective as NaCl in reversing the non-electrolyte effect and that times of considerably less than 30 seconds are sufficient to produce the change.

If it be supposed that the observed rise in the osmotic resistance of the erythrocytes during a 3-minute exposure to 0.2 M sucrose is due to a leakage of salts from cells that have lost their normal impermeability to cations, then it is extremely difficult to see how the original properties of the cells can so easily be restored by the simple treatment described above. The explanation of an ionic exchange between essentially normal cells and their surroundings seems much more plausible. It is to be noted that the later and slower increase in resistance, which we have interpreted as being due to an escape of salts, cannot be reversed in this way. This is shown not only by experiments in which the original exposure to 0.2 M sucrose is of long duration, *e.g.*, an hour, but also by the fairly rapid falling off of the figures in Table V. Evidently, even in the presence of added NaCl, the high degree of hemolysis at first produced cannot be maintained, which is what would be expected if salts slowly escape from the cells into the solution.

V

It remains to consider the manner in which a mere reversible exchange of ions between an unaltered erythrocyte and its surroundings could simulate the osmotic effect produced by an irreversible escape of salts or other osmotically active materials. One possible method by which this effect might be produced in a cell permeable to anions and not to cations was suggested in principle many years ago by Hamburger (1902) and is further discussed by Ege (1921). If, for example, SO_4'' ions from outside the cell were exchanged for electrically equivalent quantities of Cl' and HCO_3' ions from inside the cell, the internal osmotic pressure would be lowered, since the exchange would be in a 1 : 2 ratio. Though this method illustrates the possibility of a decrease in internal osmotic pressure without "leakage" and without any other change in the normal properties of the cell, it obviously cannot apply to non-electrolyte solutions in which the only ions present are $H^{\cdot}$ and OH', both of which are univalent. However, though the exchange of Cl' or HCO_3' from the cell for OH' from the solution can produce no direct osmotic effect (see Jacobs and Parpart, 1932, for evidence that $H^{\cdot}$, as such, does not enter the cell), it by no means follows that an indirect effect is impossible. It will now be shown that, as a matter of fact, such an indirect effect is to be expected.

(In this connection see also Jacobs, 1932, and, for an important contribution to the general theory, Netter, 1928.)

It is almost universally admitted (for a summary of the evidence see Jacobs, 1931) that, whatever may be true of the erythrocyte in abnormal surroundings, in the body it is freely permeable to at least most anions and practically impermeable to cations. It is also generally agreed that of the total base contained within the cell, a part under usual conditions is combined with hemoglobin to form a dissociated salt-like compound, and most of the remainder is paired with inorganic anions such as Cl' and HCO_3'. The proportions of the total base bound by hemoglobin, on the one hand, and by other acids, on the other, are subject to rather wide variations with changes in pH, temperature, oxygenation of the hemoglobin, etc.; these variations are of importance in connection with the normal physiological transport of CO_2 by the blood. When we add the further well-established fact that the osmotic pressure of a solution of the salt of hemoglobin, which may be represented as BnHb, is much less than that of a solution containing the same amount of base in the form of a uni-univalent salt, *e.g.*, of n BCl or n $BHCO_3$, we have all the facts necessary for a plausible theory as to the apparently peculiar behavior of the erythrocyte in non-electrolyte solutions.

To avoid unnecessary complexity we may consider the system:

$$HHb + BHb + BCl \mid \text{non-electrolyte}$$

in which all the inorganic salts of the erythrocyte are for simplicity treated as chloride. The system is otherwise supposed to have the general properties just mentioned. By hypothesis, the membrane which separates the two solutions is permeable to the anions Cl' and OH' but not to cations nor to hemoglobin in any form. Under these conditions, by the principle of Donnan, the equilibrium

$$\frac{[Cl] \text{ inside}}{[Cl] \text{ outside}} = \frac{[OH] \text{ inside}}{[OH] \text{ outside}} = \frac{[H] \text{ outside}}{[H] \text{ inside}}$$

will tend to be established. Following the usual approximate treatment of such problems, we shall consider the quantities within the square brackets to represent concentrations rather than activities, from which they do not here differ very greatly numerically.

In the attainment of the equilibrium condition there must be an exchange of OH' from the external solution for Cl' from the cell. But such an exchange leads to the reaction:

$$\begin{array}{r|ll|l} HHb & & \rightarrow K\cdot Hb' & \\ K\cdot & H\cdot & H_2O & H\cdot \\ Cl' & OH' \leftarrow & & Cl' \end{array}$$

which evidently diminishes the internal osmotic pressure. No salt has left the cell and the ions Cl′ and OH′ have been exchanged in amounts which are exactly equivalent osmotically, and yet the somewhat paradoxical result appears that the cell has undergone an osmotic change in the same direction as if it had suffered a leakage of salts.

VI

It would seem that there could be little theoretical objection to the mechanism just suggested, which is deduced entirely from generally accepted properties of the erythrocyte. The only question likely to be raised is as to whether the magnitude of the effect so produced could be great enough to account for the observed initial behavior of the erythrocyte in non-electrolyte solutions. This question may be at least partly answered by a simple semi-quantitative treatment of the equilibrium in question. It is unfortunately not yet possible to make calculations of completely quantitative exactness; this difficulty is due both to the complexity of the erythrocyte and to the insufficiency of the existing data. Nevertheless, at least the general principles governing all such systems will emerge from the calculations about to be made.

For simplicity let us start with what initially amounts practically to a unit volume of cell contents, exclusive of the "osmotic dead space" referred to above, *i.e.*, with a quantity of cells containing a unit amount of water. The volume of the cell water, which may be represented by V_c, will vary during the course of the experiment with changes in osmotic pressure within the cells. The amount of the external non-electrolyte solution is assumed to be so large that its volume, V, will undergo no appreciable changes. This condition is actually realized in the experiments described above in which the cells were suspended in over 1000 times their own volume of solution. The external solution at the beginning of the experiment will be assumed to be exactly neutral and isosmotic with the blood. Cases where it is not initially isosmotic can be treated by imagining that before any other changes occur the cells gain or lose enough water to bring them into temporary osmotic balance with the surrounding solution; this treatment is justified because we are dealing with a final equilibrium and the position of such an equilibrium is not affected by the order in which the various supposedly reversible changes that lead to it occur.

At equilibrium we must have:

$$\frac{[\mathrm{Cl}]\ \text{inside}}{[\mathrm{Cl}]\ \text{outside}} = \frac{[\mathrm{H}]\ \text{outside}}{[\mathrm{H}]\ \text{inside}}. \qquad (1)$$

For convenience $H^{\cdot}$ ions rather than OH' ions have been used in this equation because, although the cell is believed to be impermeable to them, their concentration at all times bears the fixed relation to that of OH' represented by $[H][OH] = K_w$. There may now be substituted for the four quantities in brackets in equation 1 expressions involving V_c and V, already defined, and the amounts of hemoglobin, a, total base, b, base bound by hemoglobin, b', and chlorine, c, initially present in the cell, together with the amount, x, of Cl' exchanged during the course of the experiment for OH'. The resulting equation may then be solved for x and the magnitude of the resulting osmotic effect estimated.

The equilibrium value of $[Cl]$ inside is obviously $(c - x)/V_c$. But the volume of liquid in the cell is supposed to be determined according to ordinary osmotic laws by the quantities of solutes present. If the initial volume is unity, V_c must therefore be equal to $(a + b + c - x)/(a + b + c)$. It will be noted that a and b undergo no change, since the cell is impermeable to hemoglobin in all forms and to cations; x therefore represents the total change in the solutes in the cell. The value of $[Cl]_{outside}$ is the simplest of the four quantities in brackets, being merely x/V. Of the quantities on the right-hand side of the equation, $[H]_{outside} = [Cl]_{outside} + [OH]_{outside}$, or, substituting x/V for $[Cl]$ and $K_w/[H]$ for $[OH]$ we have:

$$[H]_{outside} = \frac{\sqrt{K_w + \frac{x^2}{4}} + \frac{x}{2}}{V}.$$

For practical purposes, since K_w in our problem is very small compared with $x^2/4$, no appreciable error is caused by placing $[H]_{outside} = x/V$. This is the same as saying that $[H]_{outside}$ is practically the same as $[Cl]_{outside}$.

We have now only to find the value of $[H]$ inside in terms of the quantities already used in order to have a complete equation from which x may be determined. Fortunately, the reaction $HHb + K^{\cdot} + OH' = K^{\cdot} + Hb' + H_2O$ is practically complete within the pH range dealt with here, so x, the amount of OH' that enters the cell, is with no appreciable error, equal to the additional base combined with hemoglobin over and above the amount, b', initially present. For the relation between pH and the amount of base combined with hemoglobin we have selected as sufficiently accurate for our purposes, the simple equation given by Van Slyke, Hastings, Heidelberger, and Neill (1922) in place of their more complicated one published later.

The equation used is that for oxyhemoglobin, since oxygenation is almost complete under the conditions of our experiments. It is:

$$\text{Total base combined} = b' + x = 2.6\ \text{Hb}\ (\text{pH} - 6.6). \qquad (2)$$

This may be rewritten

$$\text{pH} = \frac{b' + x}{2.6\ \text{Hb}} + 6.6$$

and finally,

$$[\text{H}]_{\text{inside}} = 10^{-\left(\frac{b'+x}{2.6\ \text{Hb}} + 6.6\right)}.$$

Conditions within the erythrocyte are doubtless somewhat different from those for which this equation was intended to apply, but probably not sufficiently so to deprive our calculation of at least a semi-quantitative significance. Though the equation describes the behavior of hemoglobin at 38° C. and our experiments were made at 20° C., this difference is not as serious as it might appear to be, since we are interested primarily in *changes* in base bound by hemoglobin and, according to Stadie and Martin (1924), the changes in BHb with change in pH, *i.e.*, the buffer value of hemoglobin, are scarcely affected by moderate temperature changes.

Combining now the values separately found for the four quantities in brackets in equation (1) we have, after transferring $[\text{H}]_{\text{outside}}$ to the left-hand side:

$$\frac{x^2(a + b + c - x)}{V^2(a + b + c)(c - x)} = 10^{-\left(\frac{b'+x}{2.6\ \text{Hb}} + 6.6\right)}. \qquad (3)$$

By the substitutions of appropriate numerical values for a, b, c, V and b', equation 3 may be solved for x by any of the usual methods for the solution of transcendental equations (see in this connection Scarborough, 1930). The values here chosen have mostly been taken in round numbers from the data given by Henderson (1928, pages 195–6) after reducing them to amounts per unit volume of cell water. For b we have taken 0.160 and for c, 0.080 mols per liter of water. For total hemoglobin in the equation used for the calculation of combined base, we have taken 0.030, which is approximately Henderson's figure, and is measured in terms of the combining power of hemoglobin for oxygen; for a, on the other hand, which is a measure of the osmotic effectiveness of the total hemoglobin, a lower figure is necessary and we have somewhat arbitrarily chosen one of 0.010; the exact value assigned to a is, as a matter of fact, within reasonable limits, of little consequence. The calculated amount of base combined with hemoglobin, b', at pH 7.1, the assumed initial intracellular pH, proves to

be somewhat less than Henderson's figure, but to be consistent we have used equation 2 throughout. The value so calculated is 0.039.[1]

In Table VI are given the calculated equilibrium values of x and V_e and of the concentrations of Cl′ and H′ inside and outside the hypothetical simplified erythrocytes for two selected values of V. The larger one represents approximately the conditions of the experiments where one drop of blood was suspended in 25 cc. of solution. It is obvious that under these conditions the osmotic effect of the ionic exchange in question is very considerable. The calculated volume change in Table VI for $V = 1000$ is approximately 15 per cent. This change would be more than enough to account for the rapid initial decrease in the percentage of hemolysis shown in Table IV, since in

TABLE VI

Equilibrium values calculated by means of equation 3 for the two cases: $V = 100$ and $V = 1000$.

	$V = 100$	$V = 1000$
x	0.00697	0.0368
V_e	0.972	0.853
$[Cl]_o$	6.97×10^{-5}	3.68×10^{-5}
$[Cl]_i$	7.51×10^{-2}	5.07×10^{-2}
$[Cl]_i/[Cl]_o$	1.08×10^{3}	1.37×10^{3}
$[H]_i$	6.47×10^{-8}	2.68×10^{-8}
$[H]_o$	6.97×10^{-5}	3.68×10^{-5}
$[H]_o/[H]_i$	1.08×10^{3}	1.37×10^{3}

the preliminary standardization of the blood used in this experiment it was found that an increase in concentration from 0.125 M to 0.135 M sucrose (*i.e.* about 8 per cent) reduced the percentage of hemolysis by about the same amount as the 3-minute pre-hemolytic exposure to 0.2 M sucrose.

It should be strongly emphasized that no importance is attached

[1] This value of b' gives rise to the difficulty that the sum of b' and c is not equal to b as it should be. However, even if this equality had been preserved by reducing b to 0.119, the effect on Table VI would have been so slight as to be scarcely noticeable; the new values of x in this case would have been 0.00699 and 0.0372 and those of V_e 0.967 and 0.822 instead of the ones given. From the manner in which b enters into equation 3, its exact value over a considerable range, like that of a, is relatively unimportant.

by us to anything but the order of magnitude of the calculated values of x and V_e. In the first place, the hypothetical system for which the calculations have been made differs from the erythrocyte in several important respects; for example, the CO_2-bicarbonate system has purposely been omitted from it. Furthermore, it is impossible under actual experimental conditions to expose erythrocytes to pure non-electrolyte solutions—at least without first altering them by excessive washing, since otherwise some salts will of necessity accompany them —and Table VI as well as the experiment on which Table V is based show the important effect of very low concentrations of electrolytes in the external solution. Finally, there is the difficulty, discussed in an earlier paper (Jacobs, 1932) that even during the process of hemolysis in a non-electrolyte solution a change in osmotic resistance may occur; and for this reason the degrees of hemolysis observed in the controls may be affected in a way that is far from simple. It would seem impracticable, therefore, at least by the present type of experiments, to make a thoroughly satisfactory quantitative test of the correctness of the theory here advanced. We believe, however, that the calculations that have been made, rough as they admittedly are, will at least serve to indicate that a reversible ionic exchange is not unworthy of further consideration as one of the factors responsible for the apparently anomalous osmotic behavior of the erythrocyte.

We are very glad to acknowledge the valuable assistance of Mr. Samuel A. Corson in connection with many of our experiments.

Summary

1. The degree of hemolysis of ox erythrocytes produced by a given hypotonic NaCl solution is not decreased by a previous exposure of from a few seconds to several hours to a similar but slightly less hypotonic non-hemolytic solution. Within this period the hemolysis method gives no evidence of any escape of osmotically active substances from the cells. After 3 or 4 hours in the case of ox erythrocytes, and from a few minutes to an hour in the case of those of the pig, cat, rabbit and man, there may be a gradual increase in osmotic resistance suggestive of a slow escape of salts.

2. Solutions of sucrose, either hypotonic or isotonic, cause a striking increase in the osmotic resistance of erythrocytes; this occurs in two stages: a rapid one and a much slower one which follows the first after a considerable interval. While the slower change is probably associated with an escape of salts, the more rapid and more striking one, which is readily reversible, is interpreted as being due to an exchange of anions from the cell for OH' ions from the solution, the

resulting increase in base bound by hemoglobin causing a decreased osmotic pressure within the cell. Calculations show that the expected osmotic effect of such an exchange is of the order of magnitude of that actually observed.

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NOTES ON THE CENTRIOLES OF AMPHIBIAN TISSUE CELLS

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INTRODUCTION

The central apparatus was a favorite object of cytological study for about thirty years following its discovery by Van Beneden in 1876. The extensive literature on this subject during that period included a considerable body of data tending to the conclusion that a part of the central apparatus, the pair of centrioles, is a constant component of most metazoan cells. These data, chronologically tabulated by Heidenhain in 1907, form an impressive array including examples of centrioles in many invertebrates and in practically every type of vertebrate somatic tissue cell. I have recently been able to confirm many of these results in studies on the favorite object for histological researches, the tissues of Amphibia; and in view of the fact that the earlier results have received practically no detailed attention in American cytological literature it seemed to me that it might be worth while to describe my observations briefly.

The greater part of the literature on centrioles has been concerned entirely with the behavior of these bodies during mitotic cell division. Curiously enough, researches demonstrating that the centrioles found in non-dividing cells actually separate and take positions at the ends of the mitotic spindle are rather rare. Accordingly I have included in Part II a description of a very clear case of this sort.

MATERIAL AND METHODS

The centrioles of non-dividing cells have all been drawn from serial sections of a 14 mm. larva of *Ambystoma opacum*. By this stage the animal has been feeding for some time, yolk resorption is completed, and most of the tissues are essentially like those of the adult. The animal was fixed in Benda's fluid, mordanted, and stained by the alizarin-crystal violet method. The slides were differentiated especially for demonstration of centrioles, which are stained an intense violet by this method. In such a preparation only the following cytoplasmic structures are stained in addition to the centrioles: myofibrillæ of striated muscle fibres, prozymogen granules of the pancreas.

granules in the cells of the first part of the proximal segment of the pronephric and mesonephric tubules, and the eosinophilic granules of leucocytes. Accordingly, in most cells the cytoplasm is almost completely colorless and homogeneous except for the two deep purple centrioles. Occasionally the chondriosomes are visible though unstained. But in most cells, although these bodies are well preserved, as can be demonstrated by heavier staining with the crystal violet, the chondriosomes are indistinguishable when unstained, presumably because their refractive index is almost exactly that of the general cytoplasm. I have made all the drawings from this one animal for the very obvious reason that such a method is of itself almost a completely adequate refutation of any doubt as to the homology of the centrioles in the different tissue cells. Indeed on any one section there are always examples of centrioles in a considerable number of different cells.

The study of the leucocyte divisions (Part II) has been made from the leucopoietic capsule of the liver of *Amphiuma tridactylum* also prepared by the Benda method.

Observations

I. Centrioles of Non-dividing Cells

Connective Tissues.—The fibroblast (Fig. 1) contains a pair of centrioles in a small volume of cytoplasm closely adjacent to the nucleus. This agrees with the observations of Spuler (1896) on the mammalian fibroblast. The cartilage cell (Fig. 2) contains a pair of large centrioles either near the surface of the cell as here figured or in an internal position near the nucleus. Wherever located the centrioles are surrounded by an area of cytoplasm that stains more heavily than the exceptionally clear remainder of the cell. This region is obviously the same as the more sharply demarcated sphere of cytoplasm described by von Smirnow (1906) in the cartilage cells of *Proteus*.

Smooth Muscle.—This cell, of which only a part is shown in Fig. 3, contains a pair of small centrioles located opposite a point approxi-

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EXPLANATION OF PLATES I–VI

The figures are all camera lucida drawings made at the table level with a Zeiss 120 objective and a 20× ocular. This gives a magnification of approximately 3600 diameters. The figures have been reduced one-half in reproduction. The epithelial cells of Plates II and III have all been mounted so that the lumen end of the cell faces the top of the plate. The figures of Plates I, II, and III have been drawn from serial sections of a 14 mm. larva of *Ambystoma opacum* prepared by the Benda method. Plates IV, V, and VI were drawn from slides of the liver of *Amphiuma tridactylum* prepared by the same method.

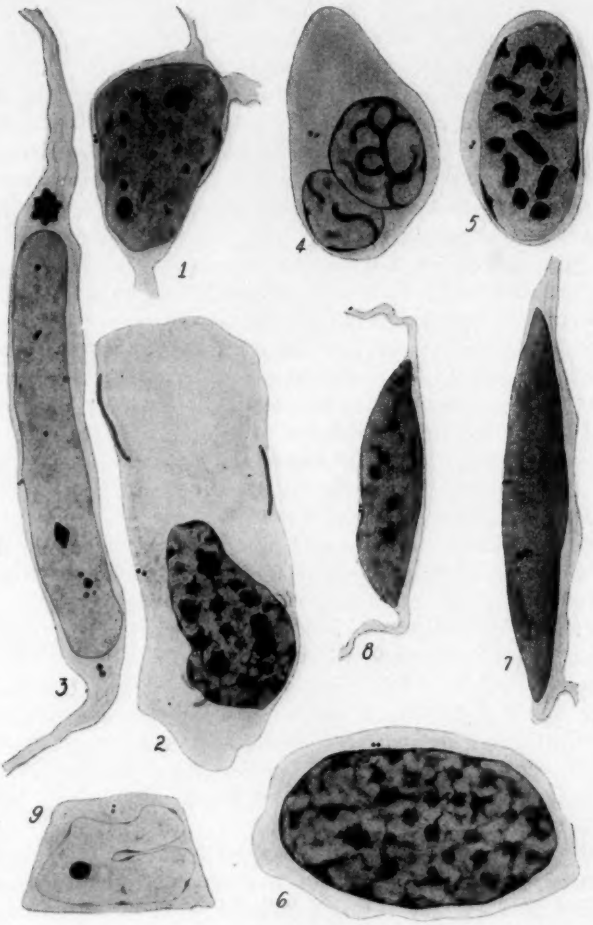

PLATE I

Explanation of Figures

1. Fibroblast from the submucosa of the wall of the stomach.
2. Cartilage cell from the basisphenoid cartilage.
3. Smooth muscle fibre from the wall of the stomach.
4. Non-granular leucocyte from the capsule of the liver.
5. Thrombocyte from the atrium.
6. Epithelial cell from the serosa of the stomach.
7. Epithelial cell from the lining of the pericardium.
8. Endothelial cell from branchial vessel.
9. Cell from the lower layer of the stratified squamous epithelium lining the pharynx.

mately midway along the length of the nucleus, where there is frequently a small depression in the nucleus. This observation agrees exactly with the results of von Lenhossék (1899).

Blood Cells.—The non-granular leucocyte of *Ambystoma opacum* (Fig. 4) has a nucleus that is roughly kidney-shaped and located in one side of the cell. The two centrioles lie nearly in the center of the cytoplasm opposite the concave surface of the nucleus. By study of other preparations it becomes clear that the centrioles are enclosed in a sphere as in *Amphiuma* (Fig. 26). The leucocyte centrioles have been more extensively studied than those of any other vertebrate tissue. Heidenhain (1907), who has given them most detailed attention, frequently found cells that apparently contained three centrioles. I have never seen any cell that had more than two and I think that Heidenhain's results can be completely understood when one realizes that he used the iron hematoxylin method of staining centrioles. As shown in Fig. 26, closely adjacent to the centrioles there is a third body, which I have somewhat inaccurately called a centrosome (Pollister, 1932*a*), and which is the focal point of the rays of the large aster which is characteristic of amphibian leucocytes. This centrosome often stains as heavily with iron hematoxylin as do the two centrioles and in my slides prepared by this method one sees a majority of the cells with three granules in the position of the centrioles. With the Benda method, however, it is easily seen that one of these is the centrosome and two are the centrioles since only the latter are stained by the crystal violet.

The thrombocyte (Fig. 5) of *Ambystoma opacum* contains a pair of small centrioles opposite the middle of the nucleus. I have been unable to identify the centrioles in the erythrocyte owing to the fact that the entire cytoplasm of this cell stains very heavily with the crystal violet.

Epithelial Tissues.—The peritoneal epithelial cell (Fig. 6) usually contains a pair of centrioles not far to one side of the nucleus, exactly as figured by Flemming (1891) in the first observations of centrioles in somatic cells. In some instances (Fig. 7) these bodies are located between the nucleus and the free surface of the cell adjacent to the cœlomic cavity. Endothelial cells (Fig. 8) have a pair of centrioles located between the nucleus and the lumen of the blood vessel.

The surface cells of the stratified squamous epithelium lining the pharynx of *Ambystoma opacum* (Fig. 10) contain a pair of centrioles surrounded by a clear sphere and located just within the lower part of the cuticle at the free surface of the cell. The deeper cells (Fig. 9) likewise have the centrioles situated between the nucleus and the

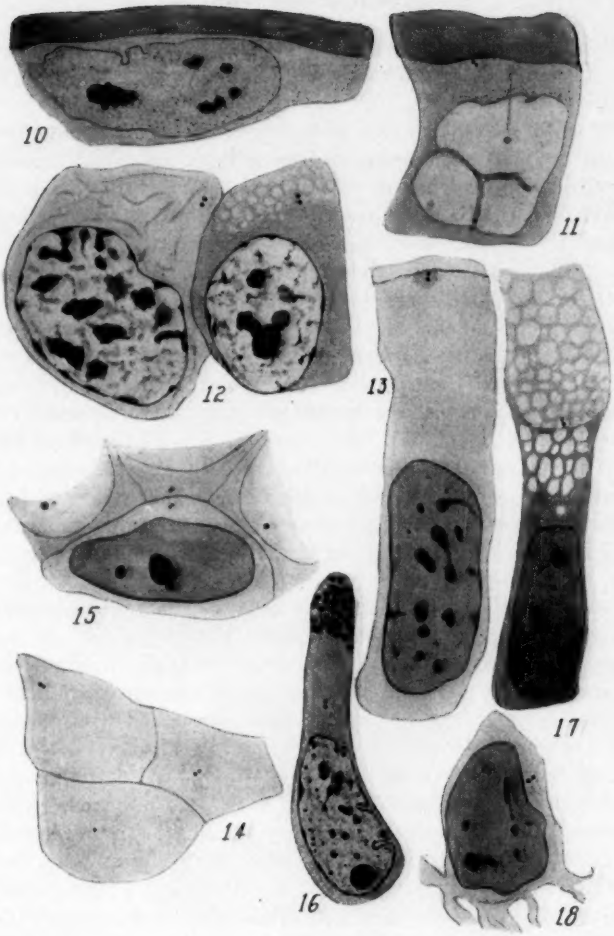

PLATE II

Explanation of Figures

10. A surface cell of the stratified squamous epithelium lining the pharynx.
11. Cell from the surface epithelium of the stomach.
12. Two cells of a gastric gland.
13. A cell from the surface epithelium lining the intestine.
14. Three cells of the surface epithelium of the small intestine seen on end from the direction of the lumen of the intestine.
15. Section of an intestinal gland showing all of one cell and parts of three others.
16. Mucous cell from the œsophagus.
17. Goblet-cell from the small intestine.
18. Epithelial cell from the lining of the unexpanded larval lung.

direction of the free surface of the epithelial layer. Both these observations agree exactly with those of Zimmerman (1898) and Joseph (1903).

The surface epithelial cells of the stomach (Fig. 11) have the end adjacent to the lumen specialized as a thick cuticle. Immediately under this cuticle is a pair of centrioles so arranged that one is always nearer the nucleus than the other. There are two types of cells in the gastric glands of *Ambystoma opacum*. These two are drawn in Fig. 12. Each cell contains a pair of centrioles between the nucleus and the lumen end of the cell, somewhat nearer the latter.

The surface epithelial cells of the intestine (Fig. 13) have a specialized border on the free surface and a pair of centrioles in exactly the same topographical relationship as is found in the gastric mucosa. This situation agrees with the descriptions given by Zimmerman (1898), and by Heidenhain (1907). As shown in Fig. 14 the centrioles are located approximately in the center of the tip of the cell in most cases. The intestinal gland cells (Fig. 15) contain a pair of centrioles surrounded by a small sphere of more lightly-staining cytoplasm and located in the same region of the cell as the centrioles of the gastric glands.

In mucous cells the centrioles are always situated deep within the cytoplasm, some distance from the lumen end of the cell—in marked contrast to the other cells in direct contact with the digestive cavity. They are generally near the proximal end of the mass of mucus that occupies the tip of the cell as previously described by Zimmerman (1898), Lenhossék (1898), Joseph (1903), and Tschassownikow (1914). A mucous cell from the oesophagus is shown in Fig. 16 and the typical goblet-cell of the intestine is illustrated in Fig. 17. I have been unable to confirm Joseph's description of a fibre running toward the lumen end of the cell from the distal centriole and another fibre extending toward the nucleus from the proximal centriole. It is interesting to note that Heiderich (1910) has seen these goblet-cell centrioles in freshly teased, unfixed preparations of frog tissues.

The cell of the peculiar epithelium of the unexpanded lung of the larval *Ambystoma opacum* (Fig. 18) has a pair of centrioles between the nucleus and the lumen, as in the other endodermal cells that have been described above.

Flagellated Epithelial Tissues.—In many epithelial cells the distal centriole serves as a blepharoplast to which a flagellum is attached. Figures 20 and 21 show two such cells from the pancreatic duct. These should be compared with Fig. 19 from the same region. The resemblance between the centrioles of these flagellated and non-flagel-

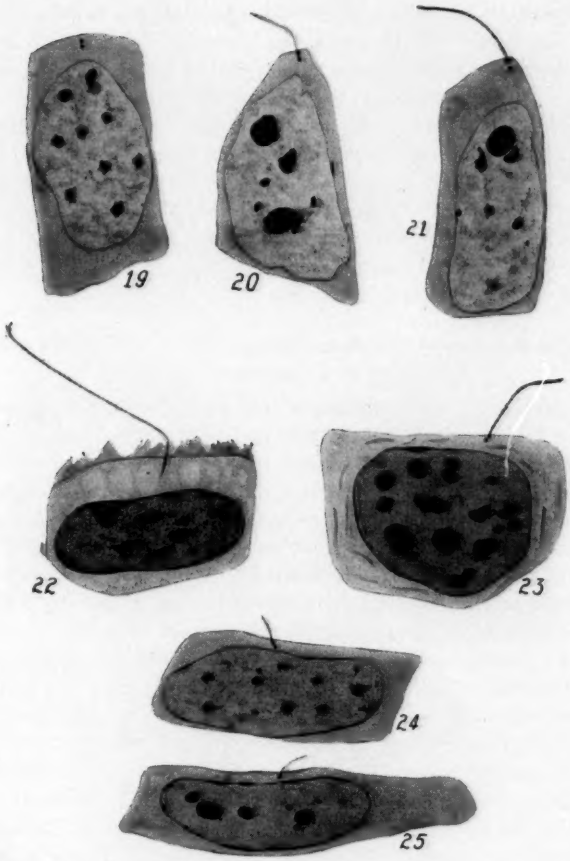

PLATE III

Explanation of Figures

19. Non-flagellated cell from the pancreatic duct.
20, 21. Flagellated cells from the pancreatic duct.
22. Flagellated cell from the proximal segment of the pronephric tubule.
23. Flagellated cell from segmental duct.
24. Flagellated cell from the distal segment of the pronephric tubule.
25. Flagellated cell from the lining of the otic vesicle.

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lated types is very convincing evidence that they are strictly homologous structures, differing only in the existence of a flagellum attached to the distal centriole in one type. Nearly every cell in the larval pronephros and mesonephros is flagellated (Figs. 22, 23, and 24). Because this study has been made from sectioned material it has been impossible to determine the characteristic length of the flagellum. The longest I have observed was more than twice the length of that in Fig. 22. This cell is of further interest as being the only instance where I have been able to find a fibre running inward toward the nucleus from the proximal centriole as described by Joseph (1903) in the ureter of *Torpedo*. In Fig. 25 is shown an example of a flagellated cell from the lining of the otic vesicle.

II. *The Behavior of Centrioles during Division of the Non-granular Leucocytes of Amphiuma tridactylum*

The production of both granular and non-granular leucocytes in the adult *Amphiuma* takes place largely in a layer of tissue, several cells in thickness, lying immediately below the peritoneal investment of the liver. As in any leucopoietic center, mitotic figures are frequent in this locality. The behavior of the centrioles during the mitosis of these cells has already been somewhat schematically figured and briefly described by Bělař (1926) in *Salamandra;* and the writer has described the present case in a short note without figures (Pollister, 1932*a*).

The centrioles of the non-dividing leucocyte (Fig. 26) are near the center of a roughly spherical mass of more heavily-staining cytoplasm, the centrosphere. In cells with kidney-shaped nuclei the centrioles-centrosome-centrosphere complex is located opposite the concavity of the nucleus as described earlier for *Ambystoma opacum* (see p. 532). The nucleus in many instances, however, is in the form of a complete ring, and in such a case the sphere lies either just outside or actually within the hole in the ring. In either case it seems likely that Heidenhain's contention that the centriole always lies approximately in the center of the cell expresses the situation quite clearly. These special leucocytes of *Amphiuma* are probably the largest found in any vertebrate. It is not infrequent to find a single cell running through as many as five sections, each six microns in thickness. In the plates, accordingly, no one cell is completely figured although in every case I have identified and studied the remainder of the cell in the adjacent sections.

The condensation of chromatin prior to actual chromosome formation has proceeded to some extent before there is any change of position of the centrioles (Fig. 27). During the later prophase stages the centrioles move apart to positions at opposite sides of the nearest

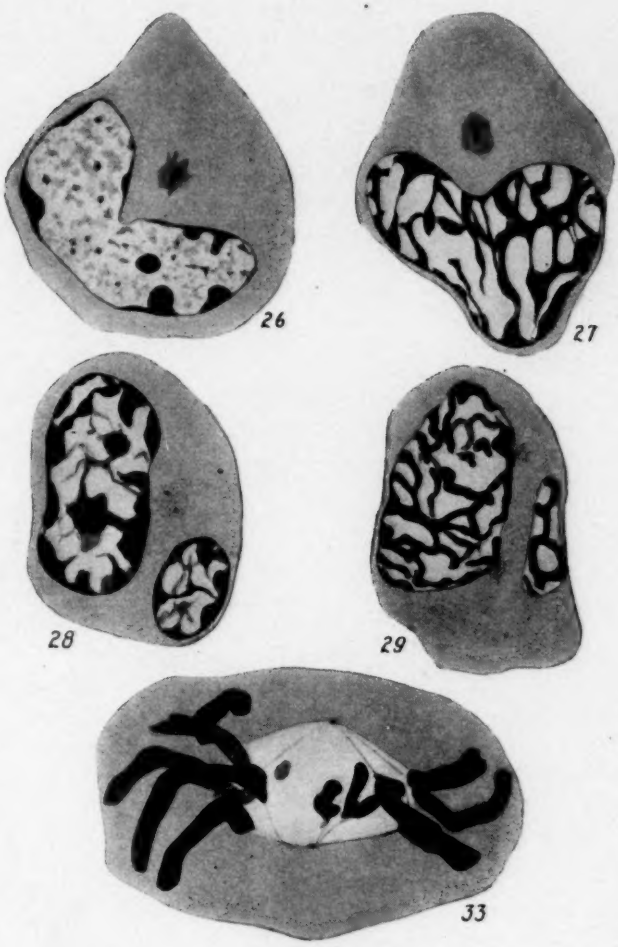

PLATE IV

Explanation of Figures

26. Non-dividing leucocyte showing sphere, centrosome, and two centrioles.
27. Early prophase. The sphere and centrosome have disappeared. Centriole migration has not yet begun.
28. Later prophase. Early stage in separation of the two centrioles.
29. Later prophase. Intermediate stage in centriole migration.
33. Metaphase stage.

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PLATE V

Explanation of Figures

30, 31, and 32. Three successive sections of a late prophase of a leucocyte with a ring-shaped nucleus. Beginning of development of the central spindle between the two centrioles.

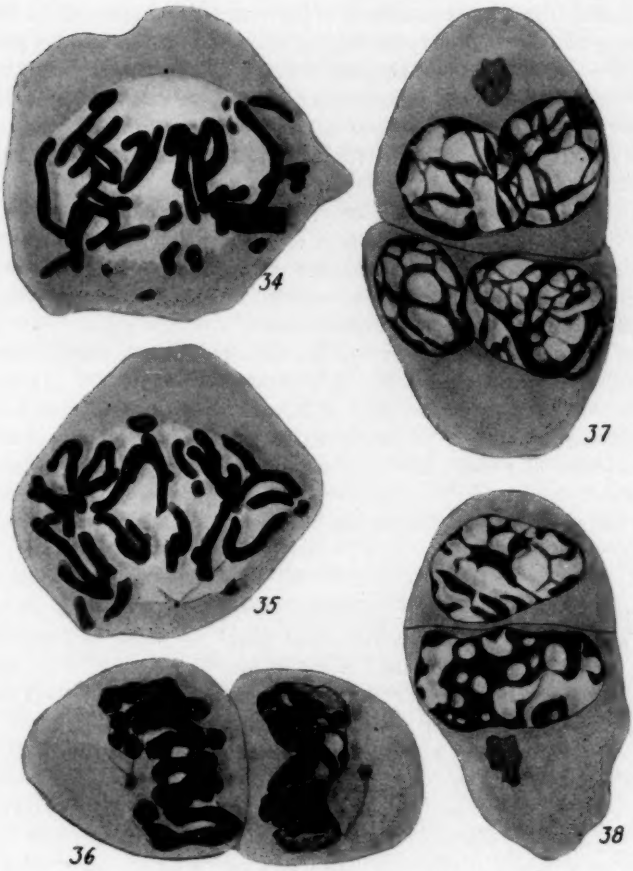

PLATE VI

Explanation of Figures

34, 35. Two sections of a cell in the anaphase stage.

36. Two sister cells in early telophase. Centrioles doubled in each cell.

37, 38. Two successive sections through two sister cells in late telophase. Sphere reconstituted about the centrioles in each cell.

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adjacent part of the nucleus (Figs. 28 and 29). In the case of a cell with a ring nucleus the centrioles migrate in opposite directions through the hole in the ring, and when this movement is completed the centrioles are no longer actually within the ring, but are located just outside opposite ends of the hole through the ring (Figs. 30, 31, and 32). At this stage, which is just before breakdown of the nuclear membrane, a spindle structure, the central spindle, appears between the two centrioles (Fig. 31). The centrioles have enlarged slightly during the prophase.

In the metaphase stage (Fig .33) the centrioles are located in the orthodox positions at opposite ends of the spindle. They are enclosed within a small, apparently homogeneous region, the periphery of which is continuous with the slender curved spindle fibres that are attached at their opposite ends to the chromosomes.

In the anaphase (Figs. 34 and 35) the centrioles are slightly smaller but otherwise they are exactly as in the metaphase.

In the early telophase (Fig. 36), just as the nuclear membrane is developing, it can first be clearly seen that the centrioles are doubled, and by late telophase (Figs. 37 and 38) a sphere is reorganized around the two centrioles in each of the sister cells and the cytoplasm has returned to a condition practically like that of the resting cell of Fig. 26.

Discussion

In the observations just described I have given examples of centrioles in a number of vertebrate tissue cells. By other workers these structures have been described in almost every other type of vertebrate and many invertebrate cells. The one outstanding exception of which I am aware is the striated muscle fibre, in which we have no clue to the presence of centrioles except the remarkable suggestion of Wolbach (1928) that in this cell the centrioles increase enormously in number and become incorporated as parts of the myofibrillæ. Personally I feel that this suggestion awaits confirmation and additional evidence before it can be seriously considered. In every case that I have studied the centrioles are two small granules that are closely adjacent to one another but morphologically independent. I agree with Joseph (1903) in being unable to convince myself of any instance where the two are connected by a fibre, the so-called centrodesmus, such as has been frequently described. I am inclined to believe that the apparent demonstration of such a structure has been due in many instances to the difficulty of getting actually clear-cut differentiation of the centrioles after staining with iron hematoxylin, a situation not encountered with the crystal violet method.

In some respects the flagellated cells are of more theoretical interest than any other type of tissue cell. They were first seen by Zimmerman (1898) in the thyroid of elasmobranch fishes (an observation more recently confirmed by Cowdry, 1921), in the uriniferous tubules of the rabbit, and in the human pancreatic duct and seminal vesicle. Meves (1899) observed the presence of a flagellum attached to the distal centriole in cells of the proximal segment of the mesonephric tubule of the salamander. It is Joseph (1903) to whom we are indebted for the most comprehensive studies of these flagellated cells as well as for the only really excellent discussion of their theoretical significance. He described and figured beautifully these cells in the epithelium of the atrium of the adult and of the entire body epidermis of the larva of *Amphioxus*, in the kidney tubules of *Torpedo*, *Salamandra*, and the rabbit, and in parts of the lining of the membranous labyrinth of the ear of mammalian embryos. In nearly every instance Joseph found a thread-like structure running inward from the proximal centriole toward the nucleus, an observation I have been unable to confirm except in one type of cell. The actual function of these flagellated cells is not known. They occur in small number among non-flagellated cells in the pancreatic duct and it is difficult to believe in this instance—or for that matter even in the larval amphibian kidney, where they occur on practically every cell—that such tiny structures could actually be of much consequence in movement of fluid along the tubule. This occurred to Zimmerman and he suggested that the flagella were actually sensory in function, serving to detect the rate of passage of material along the tubule into which they project. In that case they would presumably be non-motile. The manner in which the flagella are curved, however, certainly seems to suggest that they are actively motile.

What data we have on the ontogenetic and phylogenetic distribution of flagellated cells indicates that they are of more frequent occurrence in the lower vertebrates and in the developmental stages. I can offer confirmation of this in the fact that I am unable to find flagella in the adult urodele (*Necturus*) kidney, although here the centrioles are clear enough in their orthodox superficial position. On the whole, these facts urge me to agree with Joseph in his view that these flagella in vertebrate cells are merely vestigial structures reminiscent of a remote ancestral type of animal which had flagellated cells throughout extensive areas of epithelium lining the internal cavities of the body. One is at once reminded of the possibility of some form similar to the sponges, in which the choanocyte is essentially just this type of cell. If one accepts this view the vertebrate flagellated

cells assume considerable significance since one might regard them as one of the most primitive vertebrate tissue cells.

A further theoretical significance attaches to the flagellated cells because of the support they offer to the Henneguy-Lenhossék (Henneguy, 1897; von Lenhossék, 1898) view of the centrioles as blepharoplasts from which are developed not only flagella of epithelial cells and the axial filament of the spermatozoön but also all ciliary processes. Joseph has shown examples of cells with several flagella alongside others with a single process each. Such examples strongly suggest how, by further multiplication, a true ciliated cell might arise. The most recent and in many respects the most conclusive contribution to the Henneguy-Lenhossék hypothesis is the work of de Renyi (1924) on the tracheal epithelium of the human fetus. This author figures a very convincing closely graded series of stages in multiplication of the original two centrioles prior to the formation of ciliary processes. In its original form the Henneguy-Lenhossék hypothesis included the corollary that ciliated cells are incapable of mitotic division because of the fact that all the centrioles are, as it were, "employed" as basal bodies attached to cilia. Much of the evidence used to combat the theory has attacked this very point. Various authors (for example, Wallengren, 1905; Erhard, 1910; Saguchi, 1917) have held that observations of mitotic figures in ciliated cells or the demonstration of centrioles that are not blepharoplasts in the non-dividing ciliated cells to a considerable extent invalidate the entire Henneguy-Lenhossék hypothesis. Critical consideration is rather destructive to many of these contradictory data but even if it is granted at its face value I am unable to see why the theory of the centrioles as the only blepharoplast of vertebrate tissues is not equally plausible if merely altered to assume either that one pair of the numerous centrioles produced prior to the development of cilia remains to function as division centers, or even that a blepharoplast centriole is capable of becoming detached from the cell process and going through the orthodox behavior of centrioles at mitosis.

A most important consideration with respect to the centrioles of non-dividing cells is the question of whether they are self-perpetuating cell components. This problem today resolves itself practically into the task of proving first, that the centrioles of the non-dividing cells go to the poles of the spindle at mitosis, and second, that they are bodies persistent from a telophase of a previous division, during which they were formed by division of one of the parent centrioles. In view of the importance of obtaining crucial evidence bearing on this question, it is difficult to understand why there should be very few instances of this

sort of work. All the existent positive evidence, however, is in complete accord with the orthodox view that the centrioles reproduce themselves at cell-division. It is, of course, well known that in many instances of spermatocyte development the centriole history, including its multiplication, can be completely demonstrated from an early growth period to the spermatid. Very likely the conclusive character of this demonstration has made it seem that the addition of similar cases in somatic cells, where the task is much more difficult, was unnecessary. The evidence of the capacity for self-perpetuation possessed by centrioles in a case like that of the leucocyte described in Part II is as conclusive as is possible from study of fixed material. Tschassownikow (1914) has also demonstrated with equal clearness the complete centriole cycle in the goblet-cell. This example is particularly satisfactory because the centrioles of the non-dividing goblet-cell have been observed in fresh material by Heiderich. To me it seems that this adds the final touch necessary to make the story completely convincing. Ballowitz (1898) has described an equally good case of centriole continuity in the epithelium of *Salpa*. Heidenhain (1907) has traced out a similar complete centriole history in the mitosis of the erythrocyte of the duck embryo. Very recently Wilson and Huettner (1931) have described complete genetic continuity of the centrioles in the late divisions of the egg of *Drosophila*.

In addition to the complete demonstrations of centriole history enumerated above there are various other suggestive facts in the scanty literature on mitotic phenomena in somatic cells. An instance is the prophase phenomena in division of the pancreatic epithelium (Pollister, 1929). In gland cells, as described on p. 5, the centrioles are located between the nucleus and the lumen end of the cell, nearer the latter. In the pancreas cell this region is filled with prozymogen granules which stain heavily with crystal violet so that it is impossible to determine that the centrioles are actually present in this position. However, by analogy with other gland cells one is reasonably safe in assuming the presence of centrioles inside the mass of prozymogen granules; and this assumption is rendered more likely by the fact that in the early prophase an aster and centriole appear in exactly this region and ultimately give rise to the centrioles and asters at the ends of the spindle. There is some evidence that these centrioles divide during the later stages of mitosis, but I do not wish at this time to stress the later history of the centrioles in the division of pancreatic cells due to certain difficulties in interpretation of the telophase phenomena.[1] It is at

[1] A possible suggestion as to how this problem of returning the centriole to its orthodox position in the non-dividing cell may be solved is offered by the work of Poska-Teiss, 1933.

any rate significant that the prophase events in the pancreas cell are completely in line with other cases where the complete history of the centrioles is demonstrable.

Another interesting case is that of the mitotic phenomena of the smooth muscle cell (Pollister, 1932*b*). The early metaphase spindle is at right angles to the cell axis, apparently foreshadowing a longitudinal splitting of the fibre in the later stages. But the cleavage ultimately occurs in a plane at right angles to the long axis of the fibre because of rotation of the spindle in the later metaphase. The position of origin of the metaphase spindle, which is at first sight somewhat puzzling, is easily understood when it is realized that in the non-dividing cell the centrioles are located midway along the length of the nucleus, a condition brought about by rotation of the chromosome plate and the associated centrioles in the telophase of the last division. Apparently, when the centrioles separate in the prophase to go to opposite sides of the nucleus they migrate the least distance that will bring them into this relationship to the nucleus. This, of course, places them on opposite sides of the nucleus midway along its length, and as a consequence the spindle arising between the centrioles is at right angles to the long axis of the nucleus and of the cell.

In the aggregate it seems to me that this mass of data on the centriole history offers completely adequate support for the view that the animal tissue cells characteristically possess a pair of centrioles which perpetuate themselves at each mitotic division.

Conclusion

In concluding this short discussion of some aspects of centrioles I will briefly summarize what seem to be the essential facts concerning these components of the cytoplasm of vertebrate tissue cells. *The centrioles are minute self-perpetuating bodies characterized by somewhat specific staining reactions but more by their specific location in each type of cell and by their behavior. Centrioles show two general characteristic functions: First they have the capacity of functioning as blepharoplasts at the bases of motile cell processes—flagella or cilia. Second, in the prophase of mitotic cell-division they assume positions that determine the orientation of the spindle—an event that is the initial visible indication of the bipolarity that foreshadows division into two daughter cells.*

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